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CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 8 AUGUST, 1948 NUMBER 8

Ascorbic Acid Content in Human Cancer Tissue*

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(Received for publication June 3, 1947)

In recent years many authors have attempted to find a relationship between cancer and vitamins. There is extensive literature on this question but the varying results are often contradictory. Experiments were carried out principally on animals, whereas on human cancer there are comparatively few observations.

Several workers investigated the effect of the administration of vitamins; others, the effect of vitamin deficiency. Another group studied the vitamin metabolism of the cancer-bearing organism; still another group dealt with the vitamin content of cancer tissue. Our investigations belong to the last group, but they differ from the previous work in that they (a) refer to human cancer, and (b) compare the vitamin content of normal and cancerous tissue.

According to Watson (16) the reserves of vitamin C in the guinea pig are soon exhausted by a rapidly growing tumor. In his opinion tumor cells need vitamin C as do normal ones. Woodward (17) states that in the organs of cancer-bearing rats the ascorbic acid content does not diminish, but Sure and his associates (13), as well as Stepp and Schröder (12), found a decrease of vitamin C content in the organs of cancerous rats. Gaehtgens (6), Spellberg and Keeton (11), and others, found that patients suffering from cancer are vitamin C-deficient. However, this lack appears only in cachectic cases and is therefore only a secondary consequence, according to the explanation advanced by Góth (7) and Antes and Molo (1). Fodor and Kunos (4) assert that ascorbic acid accelerates the growth of Ehrlich tumors in mice. Gordonoff and Ludwig (10) found that vitamin C was ineffective in the culture of cancer tissue. From their experiments *in vitro,* Vogelaar and Ehrlichman (15) concluded that ascorbic acid hinders the ameboid movement of the cells and increases cell-diffusion (*see* Harding and Lech [8], also Burk and Winzler [2]).

EXPERIMENTAL

We determined the vitamin C content of human cancer tissue and of the surrounding normal tissue of the same organ by titrating with 2-dichlorphenol-indophenol the sulfosalicylic extract of tissue homogenized with glass-powder. In Harris' opinion (9), this procedure titrates not only ascorbic acid but other reducing substances such as "reducytin" as well. According to Frommel and his co-workers (5) and Voegtlin and his group (14), however, this method is adequate for ascertaining only the ascorbic acid content. We determined with this method the vitamin C content of 18 human tumors, surgical removed, and 3 other tumors taken from cadavers two hours after death (*see* cases 4, 18, 21 in Fig. 1), and the level of vitamin C of the surrounding normal tissue.

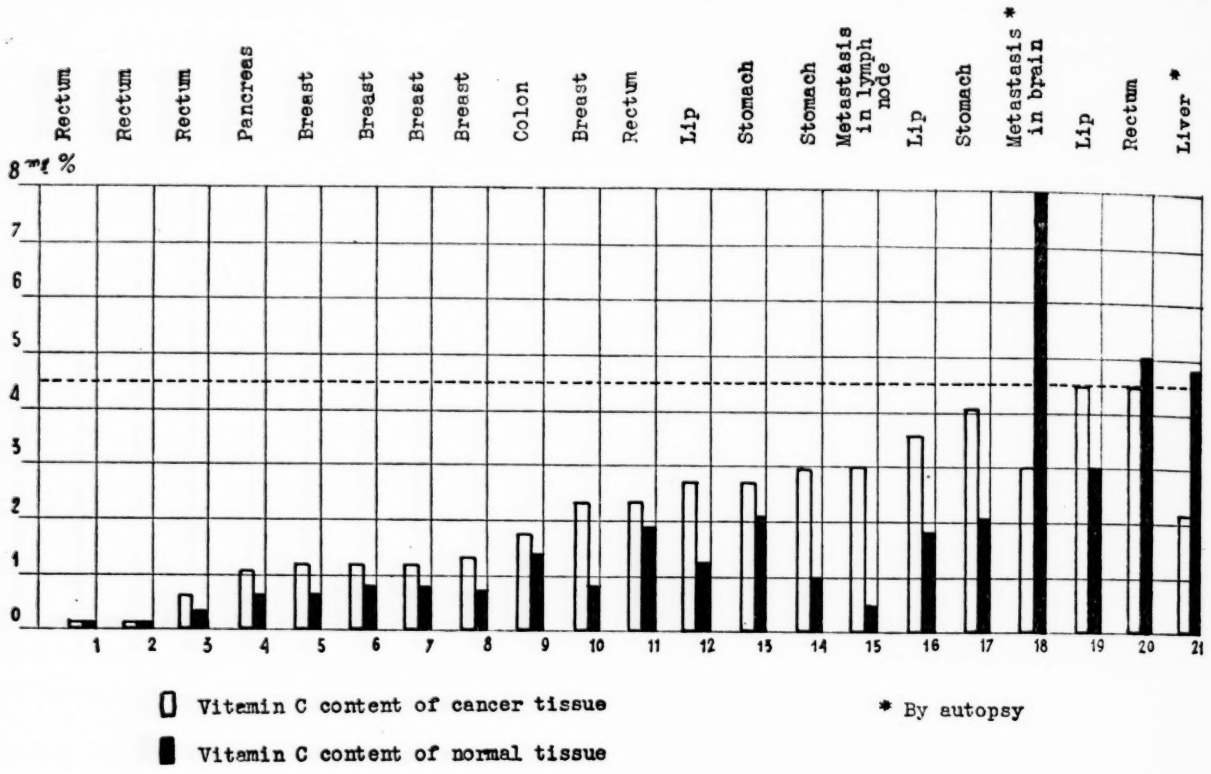
RESULTS

The vitamin C content of human tumors varied from traces to 4.5 mgm. per cent. It would appear that human cancers contain on the average 10 times less ascorbic acid than do those of animals. Woodward (17) found in Welker carcinoma and in Philadelphia sarcoma 48 to 65 mgm. per cent of ascorbic acid, whereas in Jensen sarcoma Euler and Malmberg (3) found 20 to 25 mgm. per cent. From our further investigations it has been obvious that, in general, tumors have more vitamin C content than the normal organ from which the cancer originates. We cannot give the proper explanation, but probably the tumors contain more cells than do normal tissues.

DISCUSSION

Ascorbic acid is contained in moderate quantities in many human cancers. Apparently, cancer originates frequently in organs in which the vitamin C

* Because of an accelerated production schedule, the authors have not read proof of this paper.



content is low, *i.e.*, below 4.5 mgm. per cent, but rarely grows in organs containing ascorbic acid above the level of 4.5 mgm. per cent. Eighty-eight per cent of all cancers originate from organs containing less than 4.5 mgm. per cent of vitamin C, whereas from organs containing more than this level of vitamin C only 12 per cent of cancers originate, according to Zalka's reports on 4,904 autopsies (18). Naturally the vitamin C content of the organs depends also on the intake of vitamin C but this is revealed in only a small degree by our investigations. After the siege of Budapest in 1944 when the vitamin C content of foodstuffs had been extremely poor, there was as much vitamin C in the organs as there was in the following summer when vitamin C was abundant.

The ovary probably constitutes an exception to the rule suggested above. We found 13 mgm. per cent of vitamin C in normal ovaries. However, cancer often originates from this organ. It is well known, and our histochemical research has also revealed, that in the ovary only the cells with endocrine function (follicle) contain much ascorbic acid, and cancer does not arise from these cells.

The situation is similar for the islands of Langerhans. This relationship between organic vitamin C content and cancer origin is confirmed by the fact that in the fetus and in childhood the content of vitamin C in organs is generally above 4.5 mgm. per cent, and at these ages cancer occurs rarely.

We cannot ascribe entirely the cancer-susceptibility of organs to their vitamin C content; the frequent occurrence of cancer in tissues with a low ascorbic acid level emphasizes this caution.

SUMMARY

In general, the vitamin C content of human cancer is somewhat higher than it is in the surrounding normal tissue but does not exceed the level of 4.5 mgm. per cent. Therefore, the vitamin C level of human cancer is relatively low compared with that of animal tissues. If the vitamin C content of the surrounding normal tissue is higher than 4.5 mgm. per cent, then there is less vitamin C to be found in the cancer tissue in comparison with the normal tissue.

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A Method of Staining Nucleoli of Cells in Fresh Benign and Malignant Tissues*

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(Received for publication July 16, 1947)

INTRODUCTION

With the increasing interest in the diagnosis of malignant cells in various exudates and body fluids, a method demonstrating certain nucleolar changes in considerable detail may be of value. It is the purpose of this paper to present such a method and to discuss briefly the nucleolar changes in benign and malignant tissues and also to point out the potential applicability of this method as an aid in the diagnosis of cancer.

The azure C method is based on the observation that nucleoli of cells of fresh tissues when exposed to certain dyes, stain electively. Naegeli (17, 18) states that the nucleoli of lymphocytes and myeloblasts often stain well with supravital technics as described by Pappenheim, Nakanishi, Sabrazes, Schilling-Torgau and Cesaris-Demel. These methods utilize methylene blue, brilliant cresyl blue or Giemsa stain. Quensel (21, 22), in particular, has studied the nucleoli of malignant cells in pleural and ascitic fluids using a staining mixture of methylene-blue-cadmium and Sudan III-cadmium. He states that with his method the nucleoli stain better than with any other method. MacCarty (13, 14), and MacCarty and Haumeder (15), in extensive work on the nucleoli and the nuclear-nucleolar ratio of benign and malignant cells, recommend Terry's or Unnas' polychrome methylene blue for the staining of these bodies in frozen sections. MacCarty states that the nucleolus may be better demonstrated in fresh tissues than in fixed tissues. Guzman (7, 8), in a study of the nucleoli of lymphocytes and monocytes of the peripheral blood, developed a method utilizing diluted Leishman or Giemsa stain. Von Haam and Alexander (25, 26), in their work on the cytology of malignant tumors, developed a method of staining nucleoli of fresh unfixed cells in suspension, with high dilutions (1:10,000) of toluidine blue.

In most of the methods mentioned above, methylene blue or derivatives of methylene blue have been used to produce the elective staining of nucleoli. No systematic studies have been done on the homologues of methylene blue as to their ability to stain nucleoli. In the development of the azure C method this was done. Methylene blue, azure A, azure B, azure C, and thionin were studied for their staining qualities for nucleoli in many fresh benign and malignant tissues. It was found that azure C gave the best results in that the nucleoli showed a sharper outline and increased metachromatic effects, and there was less tendency of the nucleus to be overstained as compared to the other dyes. Azure B gave almost as good results.

Azure C, or mono-methyl thionin, was first prepared by Holmes and French (10) in 1926, by the oxidation of methylene blue under acid conditions.

TECHNIC

Preparation of the stained slides.—Microslides containing a fine precipitate of azure C are prepared as follows: Solutions of azure C in 0.25 per cent and 0.50 per cent concentrations in absolute methyl alcohol are prepared. A drop of one of these solutions is placed on a clean microslide and quickly covered with another slide. The stain diffuses between the slides. Excess stain is removed from the edges with a cloth. The slides are then pulled apart with a rapid sliding motion in the long axis of the slides. The alcohol rapidly dries, leaving a fine precipitate of dye. After marking one corner of the slides with a wax pencil for identification of the stained surface, they are stored until required. Slides are made with both concentrations of the dye.

Technic for tissues—A new cut is made in fresh tissue and the surface lightly scraped with a scalpel. The small amount of tissue thus obtained is stirred into a drop of fresh human blood serum which has been previously placed on a coverslip. The tip of the scalpel is used for stirring. The coverslip is inverted over the stained slide. This results in diffusion of the cell-suspension between the two surfaces. Pressure on the coverslip is avoided. The coverslip is rimmed with vaseline to

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prevent drying. The nucleoli usually stain within a few minutes. The slide is examined under the oil immersion lens immediately after preparation. Certain precautions are necessary to obtain good results. The drop of serum should be very small so that with application of the coverslip to the slide, a very thin film of the cell suspension, which does not quite reach the edges of the coverslip, is obtained. The amount of tissue, likewise, should be very small so that the cells are somewhat flattened and no more than one cell layer in thickness. Clumps of cells are to be avoided, since the cells within the interior of the clumps do not stain. Stained slides prepared with the 0.50 per cent concentration of the dye are used for tissue. If the nucleoli do not stain, or if they stain poorly, the usual faults are: too much serum, too heavy a suspension of cells for the amount of dye, too light a concentration of dye on the slide or insufficient interval of time for staining. If the nucleus overstains, the usual faults are: too heavy a concentration of dye on the slide for the number of cells, or autolyzed tissue. For best results, tissues should be used immediately after removal from the body, since the differential staining between nucleolus and nucleus is not as distinct when old tissues are used. The best results are obtained with cells free in fluid, tissues in which the cells have not adhered to the stroma, as found in many carcinomas. The so-called "tumor milk," found on the cut surfaces of carcinomas, is especially suitable for study by this technic. Good results are not obtained with dense tissues in which cells are loosened with difficulty from the stroma. Trauma often results in distortion of the cells, and necrotic areas within a tumor are to be avoided. Several parts of a tumor should be sampled to reduce the possibility of missing the malignant portion of the lesion.

Staining of blood.—The finger is pricked and a small drop of blood is placed in the center of a coverslip which is then inverted over the stained slide. Slides prepared with the 0.25 per cent solution of dye are ordinarily used, but for leukemias a higher concentration of the dye may be required.

Staining of transudates, exudates and aspiration biopsies.—The preparations are made as described above, using the whole fluid or the sediment obtained after centrifugation, with or without dilution of the material with serum depending on its cellularity. The slides prepared with the 0.50 per cent dye concentration are used but it may be necessary to vary the concentration of the dye. In aspiration biopsies, if bits of tissue are obtained, these should be gently rubbed into the drop of serum on the coverslip to free the cells from the stroma. Bone marrow obtained by aspiration need not be mixed with serum, whereas bone marrow obtained at autopsy should be so mixed.

RESULTS

The nucleolus stains a brilliant uniformly dark azure and is in striking contrast to the relatively unstained nucleus. The outline of the nucleolus is sharply delineated; fine strands of nucleolar material may be seen between some nucleoli. Small nucleoli, which are often obliterated by heavily stained blocks of basophilic chromatin of the nucleus in fixed preparations stained with the usual methods, are well seen by this method. Vacuoles within the nucleolus are often noted. No staining distinction between plasmosomes and karyosomes is noted. The nucleus does not stain, or stains a light azure, light green, or light blue, depending on the type of cell. The cytoplasm usually assumes about the same color as the nucleolus, but may not stain or may assume a light azure to dark blue depending on the type of cell. The border often is not seen in cells removed from tissue. Staining takes place in 1 to 15 minutes.

Although the preparations are not permanent they may be kept in fair condition for several days if refrigerated. Degenerative changes vary considerably with the cell type. For example, the monocyte of the peripheral blood degenerates rapidly and there is a tendency for the nucleus to be overstained, obscuring the 2 to 5 very small nucleoli contained in this cell. In contrast, there is little tendency for the nucleus to be overstained in malignant cells and the period of profitable observation may last for hours. In general, more immature cells show less degenerative changes than do more mature cells. The cytoplasm undergoes degeneration more rapidly than does the nucleus, and the nucleus more rapidly than the nucleolus. Cells in mitosis are easily recognized. The chromosomal mass takes a light blue stain and the nucleolar fragments continue to take the dark azure stain. Individual chromosomes are not stained. Quensel (22), with his method, states that mitotic figures are not readily stained.

It has been observed in actively metabolizing benign and malignant tissues and particularly in the blood in leukemia, that after once staining, the cells may quickly become decolorized. This phenomenon has also been observed by Simpson (24), using brilliant cresyl blue, and by Quensel (22), using methylene blue. The dye is reduced to a leukobase, probably by enzymatic action of the tissue. The process is reversible and restaining will occur if one

corner of the coverslip is lifted, permitting the entrance of air to the cells. Of considerable interest is the work of Barron (1), who states that methylene blue, to which azure C is related, stimulates oxygen consumption of normal tissues, having aerobic glycolysis, and of tumors.

A detailed description of specific cells is not indicated here but a brief description of a few common cells found in serous exudates will be given.

The red cells do not take the stain. The reticulum of the reticulocytes takes a light brown stain. The small lymphocyte (Fig. 7) contains usually 1 small round eccentrically placed nucleolus and occasionally 2, but no more than 2, nucleoli. There is a thin rim of azure cytoplasm around the relatively unstained round nucleus. The monocyte of the peripheral blood contains 2 to 5 small nucleoli about half the size of those found in the small lymphocyte. The nucleus is approximately oval in shape, whereas the cytoplasm is relatively abundant and takes the azure stain. The mesothelial cell (Fig. 9) contains usually 1 small round dark azure-staining nucleolus in a round to oval nucleus and a relatively small amount of azure-stained cytoplasm. Macrophages in fluids show an oval to elongated nucleus, abundant cytoplasm that is often vacuolated, and 1 to 2 small oval to round nucleoli. They often contain ingested material and cells. Polymorphonuclear leukocytes may be recognized by the shape of the nucleus, the fine cytoplasmic granules and the absence of the nucleoli. Basophils and mast cells show bright red granules, while in eosinophils, some of the granules have a light azure tint. The plasma cell shows an oval, green-tinted, nucleus with usually 1 eccentrically placed small round nucleolus, but it may contain up to 3 nucleoli. The cytoplasm is abundant, light azure and finely vacuolated. Normoblasts show a pale blue stained nucleus. Fat does not stain. Melanin pigment of malignant melanoma stains dark blue.

DISCUSSION

Advantages and disadvantages of the method.—The advantages of the method are (a) nucleoli may be demonstrated with detail not ordinarily seen in other types of preparations using fixed and embedded tissues; (b) rapidity of the method; (c) applicability to exudates, aspiration biopsies and other types of fluid or semifluid tissues; (d) complete cells are seen and the total number and complete outline of the nucleoli may be noted, in distinction to sectioned tissues; (e) certain crystals such as Charcot-Leyden crystals, uric acid crystals or cholesterol crystals are seen (they are usually dissolved when other technics are used); (f) artefacts produced by fixation and embedding are avoided.

The disadvantages are: (a) impermanence of the preparations; (b) degenerative changes occurring in unfixed tissues with the production of artefacts; (c) in the study of malignancy, the failure to see structure of the tumor, its relation to stroma or its invasive properties; (d) sampling of only a small part of the tissue with the attendant possibility of missing the lesion. This method, however, complements other methods and should be used in conjunction with other histopathological technics.

The nucleoli of malignant cells.—The nucleolar changes of malignant cells have been studied in particular by Pianese (20), Quensel (21, 22), MacCarty (13, 14), MacCarty and Haumeder (15), Fidler (5), Castren (2), Saxen (23), Karp (11), Zadek (27), and von Haam and Alexander (26). Among the nucleolar changes in malignant cells described by these authors are: (a) increase in size of the nucleolus out of proportion to the increase in size of the nucleus; (b) irregularity in the shape of the nucleolus; (c) increase in the number of nucleoli; (d) variation in the number of nucleoli. There are two other features which the author has observed in some malignant cells: (a) the presence of fine nucleolar strands between nucleoli, morphological evidence of nucleolar division; (b) loss of polarity of nucleoli (the nucleoli are arranged at right angles to the long axis of an elongated nucleus). None of these changes are pathognomonic of malignancy. Von Haam and Alexander (26) state "neither size, shape or number of nucleoli represent any common characteristic for malignant cells." Quensel (22) states, "by my method peculiar changes in the nucleoli of malignant cells in serous exudates are so regularly found that when these changes are marked, they represent an entirely characteristic sign of malignancy." He further states that all malignant cells may not show these nucleolar changes. At present it is the consensus that there is no common pathognomonic morphologic feature of the malignant cell.

Although the nucleolar changes described above are not pathognomic of malignancy, they are of great help in arriving at a diagnosis. It has been observed that most non-neoplastic tissues and benign tumors contain from 1 to 3 small round nucleoli. However, Von Hamm and Alexander found large nucleoli in the corpus luteum cells of the ovary, and Ferreira (4) has noticed similar changes in benign tissues. He observed in the cells of the fetus, normal liver, *membrana granulosa* of the ovary and in exophthalmic goiter, an

increase in the area of the nucleolus and a decrease in the nuclear-nucleolar ratio which approximate those of cancer. In the present study, some of the nucleolar changes already mentioned have been found in rapidly growing benign tissues such as the megaloblasts of pernicious anemia, abnormal lymphocytes in infectious mononucleosis, rapidly proliferating endometrium, rapidly growing fetal tissues and fibroblasts of young granulation tissue. On the other hand, small, round, apparently benign nucleoli have been found in lymphosarcoma of the lymphocytic type, in small cell bronchogenic carcinoma and in neuroblastoma. Ludford (12) summarizes the matter when he states, "actively growing cells are characterized by their large nucleoli, and by the fragmentation and division of same. In the adult, nucleoli of cells undergoing active metabolism are relatively large; those in the 'resting' condition relatively small." It is highly probable then that the nucleolar changes herein described may be found in any rapidly proliferating tissue, of which malignant tissue is a notable example.

Application of the azure C method.—The usual histopathologic methods are adequate in the diagnosis of malignancy in tissue and are to be preferred to scraping of tumors, in which only individual cells or groups of cells are found. Even here, however, more information of the finer cytology may be obtained by means of the azure C stain. On the other hand, considerable difficulty has long been experienced in the diagnosis of malignancy in the individual cells found in various body fluids and exudates. The sediments of the fluids are embedded and sectioned with difficulty. The alternative methods of studying these cells are by the preparation of smears stained with hematoxylin and eosin, or Papanicolaou's technic (19), or the method of Dudgeon and Patrick (3), or by embedding and sectioning the sediment according to the method described by Mandelbaum (16) and Zemansky (28). Although a fairly high degree of accuracy in diagnosis may be obtained by these methods, difficulty is still experienced in the separation of macrophages and mesothelial cells from some malignant cells. In this regard, Foot (6) states, "mesothelial pleural, pericardial and peritoneal covering cells present the chief obstacle in the way of successful diagnosis as they are readily confused with tumor cells on account of their large size and prominent nucleus." It is in the differentiation of these cells from malignant cells that the azure C method is particularly applicable. With the azure C method, the mesothelial cell usually contains one small round nucleolus, as does the macrophage. In sharp contrast, many malignant cells contain large, bizarre-shaped and multiple nucleoli. The absence of these changes does not rule out malignancy completely, since the tumor must be on a cavity surface and cells must be sloughing into the cavity in order that the malignant cells may be found in the fluid. This has been previously emphasized by Helwig (9). *The nucleolar changes described above should be extensive, and should be present in many cells, to be of aid in the diagnosis of malignancy.*

At present 200 tissues have been studied at the Naval Medical School and high diagnostic accuracy has been achieved. As more data are collected they will be the subject of a later report.

The azure C method may be used for a more accurate description of nucleoli of cells. In general, nucleoli are rather vaguely described chiefly because fixed tissues and fixed smears of tissues are employed. In a fixed preparation there is precipitation of chromatin on the inner wall of the nuclear membrane in the lining network and on the outer surface of the nucleolus. This precipitation of nuclear material obscures the outline of the nucleolus which gives a false picture of size, obliterates fine nucleolar strands and may entirely hide some of the smaller nucleoli.

In frozen sections cellular detail is not well seen and it is necessary to wait 24 hours or so for the paraffin sections. With the azure C method, this difficulty may be partly obviated since considerable cellular detail may be seen at the time the frozen section is done. The azure C slide may be read often within 5 minutes, and in conjunction with the frozen section.

SUMMARY

1. A method is presented by which the nucleoli of cells of fresh benign and malignant tissues may be observed in great detail. Cell suspensions in serum are prepared on a coverslip which is then inverted over a slide containing a fine precipitate of azure C dye. The nucleoli stain clearly and electively, the nucleus does not stain deeply, permitting contrast between the nucleus and nucleolus. Advantages as well as disadvantages of the methods are described. A brief description of a few common cells as they appear with this method is given.

2. The nucleolar changes in malignant tissues and fast growing tissues are compared with those of benign tissues.

3. The value of the method as an aid in the differential diagnosis of benign and malignant cells in serous effusions is discussed.

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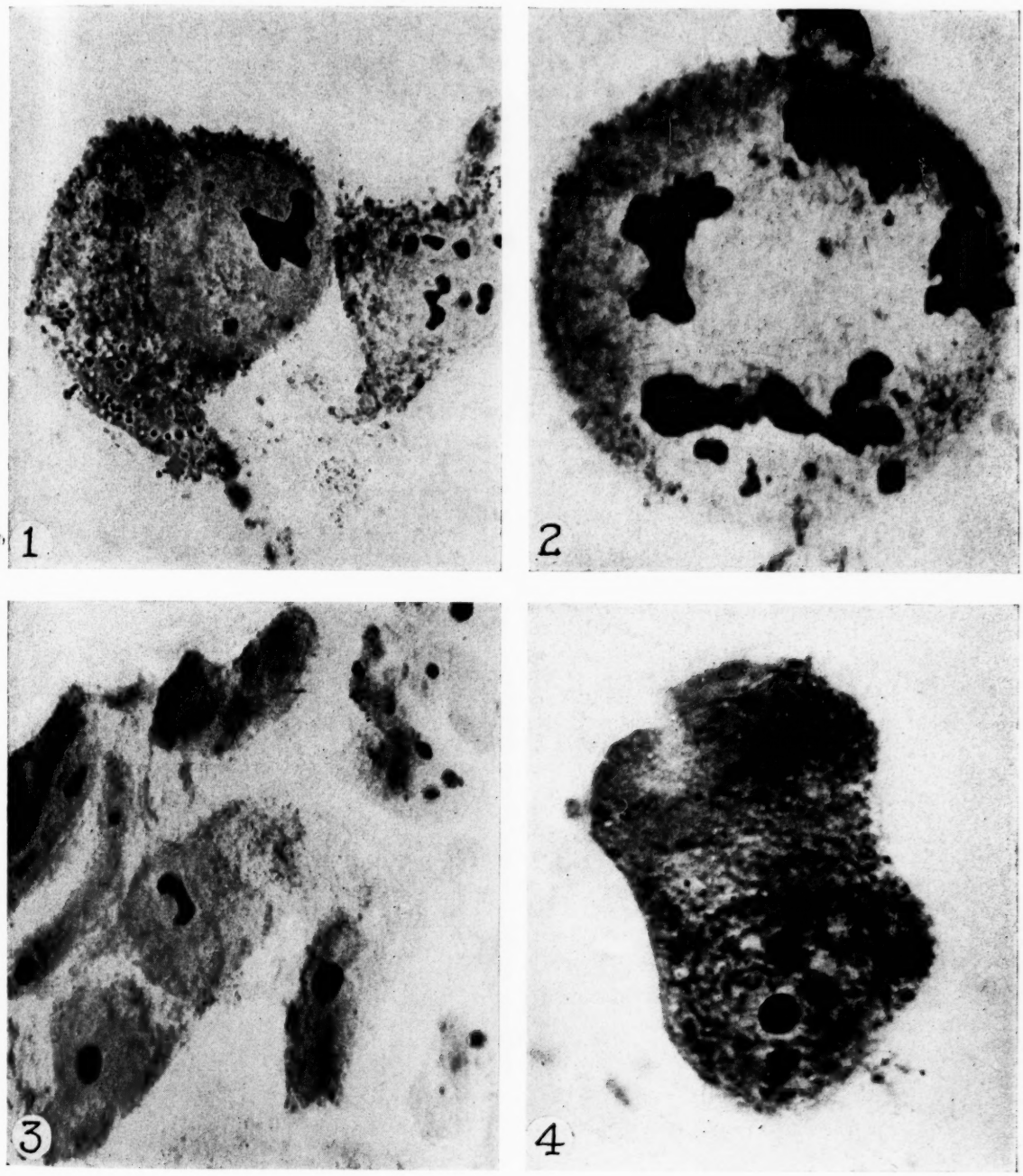
Fig. 1.—Cell of malignant melanoma. The dark irregularly shaped central masses represent nucleoli.

Fig. 2.—Cell of malignant melanoma.

Fig. 3.—Cells of adenocarcinoma of rectum.

Fig. 4.—Cell of transitional cell carcinoma of bladder. All nucleoli are not in focus. All photographs at a magnitude of 900 diameters.



FIGS. 1-4

DESCRIPTION OF FIGURES 5 TO 10

FIG. 5.—Normal cell from bronchiolar epithelium. Note the small round nucleoli.

FIG. 6.—Malignant cell from metastatic carcinoma to bone. Aspiration biopsy of lesion in scapula. Note the bizarre shape of the nucleoli and the fine nucleolar strands.

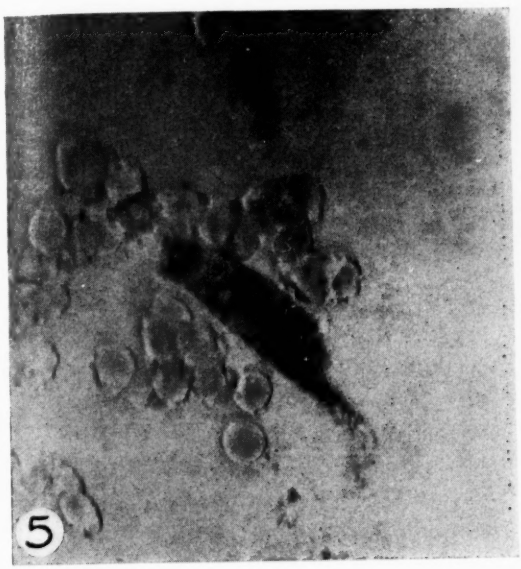
FIG. 7.—Normal mature small lymphocyte. Note the single small round eccentrically placed nucleolus. One cell is not in focus.

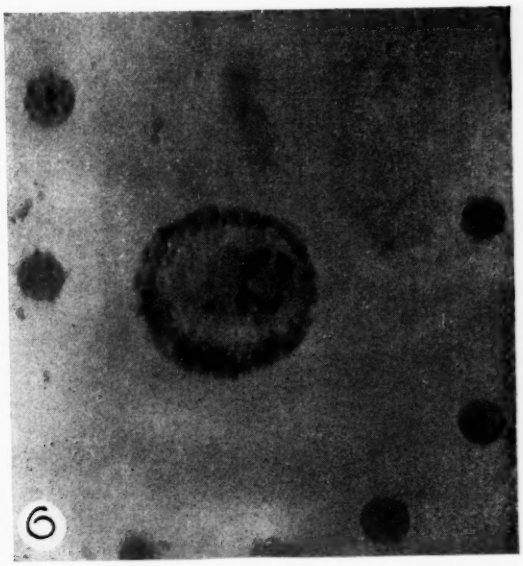
FIG. 8.—Megaloblast from a patient with pernicious anemia. Note the nucleolar strand, and the bizarre shape of the nucleolus present in a benign cell.

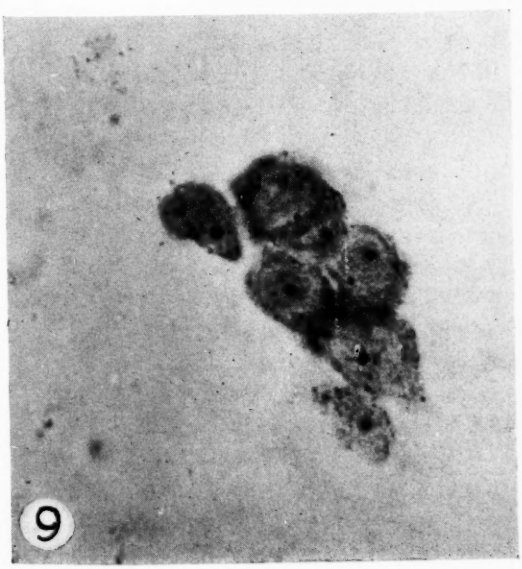
FIG. 9.—Mesothelial cells from visceral pleura of lung.

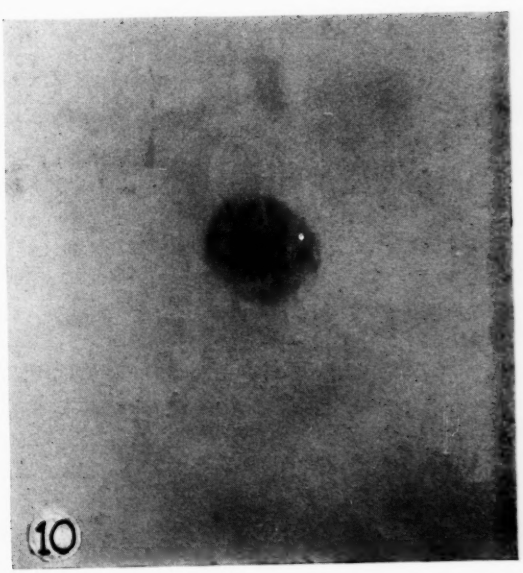
FIG. 10.—Malignant cell of adenocarcinoma of rectum, metastatic to peritoneum. In ascitic fluid.

All photographs at a magnification of 900 diameters.









FIGS. 5-10

The Diagnosis and Treatment of Primary Ovarian Carcinoma With Special Reference to Radiation Therapy

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The early diagnosis and treatment of ovarian carcinoma still remains a difficult problem due to the fact that the patient is often not seen until the neoplasm has spread beyond the ovary. Early surgical treatment has frequently proved effective when the lesion is confined to one ovary and there are no adhesions to interfere with its complete removal. The survival rate is low, however, in patients with local extension, ascites, or peritoneal implants. Roentgen therapy has proven to be of value in the relief of symptoms and prolongation of life, but there is still considerable doubt among surgeons as to whether the absolute five year "cure rate" is ever improved by radiation.

Many problems arise during the radiation treatment of these patients making it difficult and often impossible to deliver heavy dosages to the tumor-bearing areas. Radiation sickness frequently results before a course of treatment is completed due to the low tolerance of the surrounding organs. The necessity of using large fields to cover all tumor-bearing areas limits the amount that can be delivered to any single region. Factors such as the general condition of the patient, extent of the disease, presence or absence of infection, and individual sensitivity to radiation must all be considered in planning the treatment. The value of the roentgen treatment must then be determined by an analysis of the palliative effects and survival rate in the treated patients.

The records of 101 consecutive patients with ovarian carcinoma who received radiation therapy at the Hospital of the University of Pennsylvania from 1930 to 1941 have been reviewed in order to try to answer some of the problems which have arisen during their treatment and perhaps thereby improve on our methods and results in the future. Fourteen of these patients with a clinical diagnosis of ovarian carcinoma had no biopsy and are therefore excluded from this series.

Age incidence.—The age incidence parallels that reported by others (9, 22) and is shown in Table I. Seventy-two per cent of the patients are in the fourth, fifth, and sixth decades.

TABLE I: AGE INCIDENCE

Years	Patients
10–19	3
20–29	6
30–39	14
40–49	20
50–59	33
60–69	10
70–79	1
Total	**87**

Cancer history.—The question of inheritance of cancer susceptibility is becoming more important in view of the increased interest of both the layman and the physician in cancer prevention. Routine cancer studies seem somewhat impractical on a nation-wide basis at the present time. However, if cancer susceptibility, age incidence, and other factors are considered, it might become possible to carry out such survey studies in at least that group of individuals in which statistical evidence suggests the highest incidence of ovarian neoplasms. A carefully taken cancer history should become a part of every patient's record. In our group of cases, a history of cancer in the family was recorded in 20 or 35.7 per cent of 56 patients where such information was obtained, and in 16 of these 20 patients, one or more members of the immediate family had died of cancer. Lynch (10) obtained a positive history of cancer in the family in 42 of 110 ovarian carcinoma patients (40 per cent). The incidence of cancer in the family of patients with ovarian carcinoma is higher than that noted by Lynch in patients of a somewhat similar age group operated on in his clinic for non-cancerous conditions. He found a positive family history in 25.3 per cent of 1,235 women from 35 to 75 years, and 28.6 per cent of 600 women between 45 and 75 years. However, both his and our series are far too small to provide any significant data.

The development of multiple primary carcinomas

in two of our series of cases with a positive history of cancer in the immediate family further suggests a possible inherited susceptibility and emphasizes the importance of adequate follow-up examinations in such individuals. One patient developed a small lump in her left breast which was discovered during a check-up examination following her treatment for bilateral ovarian neoplasms. A simple mastectomy was performed and the pathological diagnosis was scirrhous carcinoma. A year later she detected a small lump in her right breast. This was treated by simple mastectomy and the microscopic sections revealed a small-celled carcinoma. This patient was still living and well 5 years after treatment for 3 primary carcinomas. Her sister died of carcinoma of the bowel. Another patient had treatment for breast carcinoma 2 years prior to the development of an ovarian neoplasm and is still living and well 10 years later. The ovarian lesion was discovered during a routine visit to the follow-up clinic. Her mother died of carcinoma of the breast.

Lynch (10), Meigs (14), and others (2, 18) report a lower incidence of fertility in the married women in their series. Lynch noted sterility in 31 per cent of the married women in 110 patients with ovarian carcinoma; Meigs in approximately 40 per cent of his married patients with ovarian carcinoma. Forty-two per cent of the married women in our series had never been pregnant. This is a high incidence of sterility since 10 per cent is usually considered as normal incidence (23). Many gynecologists, therefore, feel that this lack of fertility may indicate some congenital developmental abnormality of the ovary and thereby an increased chance for abnormal growth.

Symptoms and signs and diagnosis.—Ovarian cancer frequently gives no symptoms until it is well advanced. The duration of symptoms is usually from 6 months to a year before the patient consults a physician or the diagnosis is made. Pain, a sense of fullness, or a mass in the abdomen is usually the presenting symptom. Abdominal pain was present in 56 or 64 per cent of our 87 patients, while a mass or abdominal swelling was noted in 69 or 79 per cent. Twenty-one patients had noted an irregularity in their menstrual cycle and 10 had post-menopausal bleeding.

Ascites was present in 34 of the patients, though due to the large size of the tumor in some cases, this was not discovered until operation. Pleural effusion was present in 4 of these patients when they were first seen and developed later in the course of the disease in an additional 4 patients. In only 1 of these cases was there any evidence of pleural or parenchymal metastasis on repeated chest roentgenograms. The pleural effusion was apparently cured by removal of the primary tumor in 2 patients. Another patient with a bilateral pleural effusion for seven months before death had no evidence of metastasis in the chest at post-mortem examination. The pathologist was unable to explain the cause of the hydrothorax. The presence of a pleural effusion in patients with ovarian carcinoma therefore does not necessarily indicate parenchymal or pleural metastasis. The mechanism of the hydrothorax in such cases may be similar to that noted by Meigs and Cass (13) in cases of ovarian fibroma. It is interesting to note that in the 6 patients with a unilateral hydrothorax, it was present on the right side in 5, and on the left side in one patient. There was no correlation between the location of the tumor and the pleural effusion since, in the 5 patients with a right pleural effusion, the tumor was located on the right side in 2, on the left side in 2, and was bilateral in one patient. The patient with a left pleural effusion had a bilateral ovarian tumor. A greater frequency of a right pleural effusion was observed by Meigs, Armstrong, and Hamilton (15) in a report of 27 cases of ovarian fibroma. Eighteen of this group had a pleural effusion on the right side, 4 on the left, and 4 were bilateral. In one case, the location of the effusion was not stated. The effusion disappeared in all cases after removal of the ovarian fibroma.

Since the true character and extent of the lesion cannot always be ascertained from the history and physical examination alone, we believe a roentgenogram of the chest and abdomen should be obtained routinely on these patients to aid in the differential diagnosis. A urogram and gastrointestinal examination is often of value in determining the true extent of the disease and may be helpful in the future management.

Classification.—The study of pathological material has proven to be of little or no value either in predicting the radiosensitivity of the tumor or the ultimate prognosis of the patient. Harris and Payne (3), Meigs (14), Lynch (10), and others could find no real correlation between the pathological type or grading and radiosensitivity. Most authors have therefore classified their cases into groups based on the extent of the tumor at operation. We have adopted a classification similar to that of Walter, Bachman, and Harris (27), grouping the cases in four stages:

Stage I: Primary tumor completely removable,

no visible metastases.

Stage II: Extension or local metastasis present, but completely removable with primary tumor so that no visible disease remains.

Stage III: Local metastases present, but only partially removable with primary tumor.

Stage IV: Inoperable tumor; exploratory operation or removal of large tumor masses only; distant metastasis.

Using this method, our series was classified as shown in Table II.

TABLE II: CLASSIFICATION

Stage	Patients
I	21
II	12
III	35
IV	19
Total	87

Pathology.—Only those cases in whom a positive biopsy was obtained are included in this report. The pathological diagnosis made in these 87 patients is shown in Table III.

TABLE III: PATHOLOGICAL DIAGNOSIS IN 87 PATIENTS WITH POSITIVE BIOPSY

Adenocarcinoma unclassified	6
Pseudomucinous cystadenocarcinoma	9
Serous papillary cystadenocarcinoma	36
Serous non-papillary cystadenocarcinoma	30
Carcinoma arising in a dermoid	3
Granulosa cell tumor	3
Total	87

The tumor was unilateral in 43 and bilateral in 34 patients. It was located in the right ovary in 19 and in the left ovary in 24. Two patients with unilateral involvement subsequently developed a tumor in the opposite ovary 14 and 16 months later.

The extent of the tumor at the time of operation is shown in Table IV.

TABLE IV: EXTENT OF TUMOR AT TIME OF OPERATION—87 PATIENTS

Generalized peritoneal metastasis with ascites	21
Generalized peritoneal metastasis without ascites	15
Pelvic extension or metastasis with ascites	13
Pelvic extension or metastasis without ascites	16
Far extension	1
No metastasis seen	21
Total	87

Treatment.—All of the patients had at least an exploratory operation and whenever possible those operated on at our hospital and referred to the Department of Radiology from the Department of Obstetrics and Gynecology had a bilateral salpingo-oophorectomy and hysterectomy. Some of the patients referred for treatment after operation elsewhere had only a unilateral oophorectomy. The type of operation performed in this series is shown in Table V.

TABLE V: TYPE OF OPERATION—87 PATIENTS

Bilateral salpingo-oophorectomy and hysterectomy	51
Bilateral salpingo-oophorectomy	6
Unilateral salpingo-oophorectomy	11
Exploratory or removal of large tumors only	19
Total	87

All of the 87 cases reported here received postoperative radiation. Two cases were found to be inoperable at the first operation but after a full course of roentgen therapy, the tumor was markedly reduced in size so as to be removable at a second operation. One of these patients with widespread peritoneal implants at the first operation, showed complete regression of the metastatic lesions upon re-operation, after receiving a tumor dose of 1,500 tissue r to the abdomen and pelvis. The second patient showed a marked regression of the tumor mass after receiving a tumor dose of 1,500 tissue r to the pelvis, but the adbomen was not irradiated and the peritoneal transplants were still present. A third patient was found to be inoperable at the first operation and at a second exploratory operation two weeks after receiving 1,000 tissue r to the abdomen and 2,000 tissue r to the pelvis. Two months later, she was again operated on and the tumor growths were completely removed. No metastases were present at the third operation.

The two patients in whom only one ovary was removed subsequently developed a carcinoma in the opposite ovary fourteen and sixteen months later. In both cases, the opposite ovary showed no gross involvement at the first operation. One patient received no radiotherapy but the second patient had a full course of treatment following the first surgical procedure.

Radiation was given using factors of 160 to 200 kv. (constant potential), 5 to 15 ma., half-value-layer .95 to 1.1 mm. Cu, 50 to 80 cm. distance. The pelvis was irradiated through 2 anterior and 2 posterior 15 by 15 cm. or 17 by 17 cm. portals until 1941 when the technic was changed to a single anterior and posterior 15 by 20 cm. portal. Those cases with evidence of widespread peritoneal transplants were given radiation over the upper

abdomen using a single 20 by 20 cm. anterior and posterior portal. Four patients with a pleural effusion who were thought to have metastatic lesions in the chest were given palliative treatment over this region using a 15 by 15 cm. anterior and posterior portal. Patients with a large localized recurrence in the pelvis occasionally were given additional treatment over this area. Two patients with a localized lesion in the cul-de-sac received treatment through intravaginal portals in addition to the external treatment.

With the use of two anterior and posterior portals, a dose of 200 (air) r was delivered to each of two portals daily or on alternate days depending upon the tolerance of the patient. The majority of our cases were treated as outpatients and occasionally 3 or 4 portals were treated with 200 (air) r twice weekly when the patient lived some distance from the hospital. When the single large anterior and posterior portal was used, 1 portal was given 200 (air) r daily. The cyclic method was used on 10 of this group between 1937 to 1939 treating 2 portals daily for 8 to 10 days with a rest period of from 1 to 2 weeks between each cycle. There was no evidence that this method was producing any better results and it was therefore abandoned in favor of the serial method. Most patients received from 1,500 to 3,000 (air) r at each series with a rest period of from 1 to 4 months or longer between series. Those who were in good general condition and tolerated treatment well were sometimes given a full course of therapy in a single series. In some cases the total treatment was spread over 2, 3, or 4 series.

The tumor dose delivered to the mid-anteroposterior region of the pelvis and abdomen was calculated in all these patients using the depth dose charts published by Quimby (24) or Mayneord and Lamerton (12). The ovarian tumor dose and the abdominal tissue dose were taken as that delivered to the mid-anteroposterior region of the pelvis and abdomen respectively. In several patients, depth dose calculations were made in planning the treatment. In most cases, the anteroposterior dimension of the abdomen and pelvis was recorded before the start of the treatment. In a few cases, where this information was not available, on the charts, the anteroposterior dimension was determined from that recorded on the x-ray diagnostic sheets, since the majority of the patients had either a flatplate of the abdomen, urographic study or both at sometime during their treatment. Variations in the anteroposterior dimension due to reaccumulation of abdominal fluid were not considered and necessarily introduce some error in the depth dose calculations. However, when large amounts of fluid were present, this was removed by paracentesis both before and whenever necessary during the treatment. The 84 patients, in whom complete follow-ups were obtained, were grouped according to the tumor dose delivered to the pelvis as shown in Table VI.

Fifty-five patients were given abdominal radiation as well with doses varying from 200 to 2,500 tissue r, and of these 28 were given from 200 to 1,000 tissue r and 27 received from 1,000 to 2,500 tissue r. Of the patients with abdominal carcinomatosis at operation, 14 received less than 500 tissue r and only 2 of these survived more than two years, 4 received from 500 to 1,000 tissue r, 17 were given from 1,000 to 2,500 tissue r.

Results—The results of roentgen treatment are evaluated first as to relief of symptoms and second as to prolongation of life.

Symptomatic benefit was recorded in 48 of 64 patients in whom symptoms were present at the time of roentgen treatment. Fourteen patients, nearly all of whom were in very poor condition at the start of therapy received no benefit and in some instances were probably made worse. There was a noticeable reduction or complete disappearance of the mass in 27, pain was partially or completely relieved in 32, and the reaccumulation of ascites was either prevented or considerably retarded in 15 patients. A tabulation of these results are shown in Table VII.

In 7 patients the tumor recurred while the patient was still under treatment. In 2 patients the mass increased in size in spite of treatment.

The survival rate of the 84 patients in whom complete follow-ups were obtained is shown by means of a curve in Fig. 1. The general shape of

TABLE VI: TUMOR DOSE IN ROENTGENS TO THE MID-ANTEROPOSTERIOR REGION OF THE PELVIS IN 84 PATIENTS WHO HAD COMPLETE FOLLOW-UP.

Stage	Less than 500	500–1000	1000–1500	1500–2000	2000–2500	2500–3000	3000–3500	3500–4000	4000–5000	5000–6000	6000 plus	Pts.
I	2	3	1	5	4	1	1	2			2	
II	1		2	2	1	1	1	1	1	1	1	
III	4	3	7	4	6	4	2	1	1			
IV	3	3	2	2	4	2	1		2			
	10	9	12	13	15	8	5	4	4	1	3	84

the curve of survival in our series and our five year cure rate is similar to that obtained from the results reported by Kerr and Einstein (9), and Lynch (10).

Forty-five patients (53.6 per cent) died within the first 3 years and 10 of these failed to survive 6 months. Only 4 patients died between 3 and 5 years. This seems to indicate that patients who are alive at the end of 3 years have a good chance of surviving 5 years or longer.

DISCUSSION

Due to the insidious onset of ovarian tumors, the majority of the patients, 75 per cent in this series, were not seen until the disease had spread beyond the ovary. Adequate surgery is of first importance in the proper management of ovarian carcinoma. Surgery to be adequate must include the removal of both Fallopian tubes and ovaries, and uterus. In 11 of our patients only the involved ovary was removed. Two developed carcinoma in the opposite ovary 14 and 16 months later. Whether this represented a metastatic growth from the other ovary not detectable grossly at the first operation, or a new tumor, cannot be stated. Nevertheless, postoperative radiation did not appear to be as effective in this group and only 2 were living, 1 with disease, at the end of 5 years.

Table VII: Relief of Symptoms as Recorded on Histories of 64 Patients

	Symptoms Present Before Roentgen Treatment	Relief of Symptoms After Roentgen Treatment
Number of patients	64	48 (75%)
Distribution of symptoms in the 64 patients:		
Mass	51	27 (53%)
Pain	46	32 (70%)
Ascites	34	15 (44%)

Norris and Murphy (19) found microscopic evidence of malignancy in an otherwise grossly normal appearing second ovary in 7 (17.5 per cent) of 40 patients who had a bilateral oophorectomy for ovarian carcinoma. In the other 33 of their cases both ovaries were microscopically and macroscopically malignant. Our observations serve

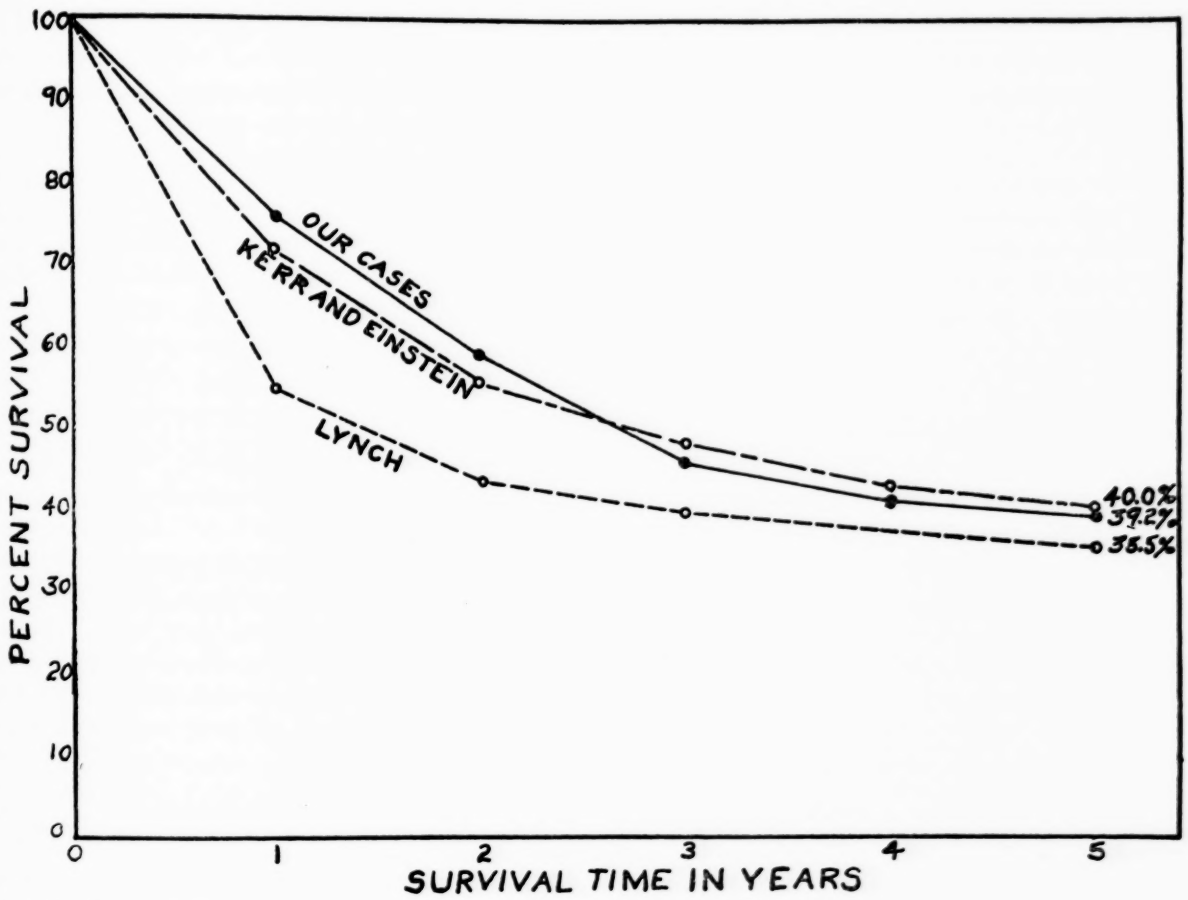

Fig. 1.—Five year survival curve in our 84 completely followed-up cases of primary ovarian carcinoma after combined surgical and postoperative radiation treatment as compared with the results reported by Kerr and Einstein (9) in 100 cases and by Lynch (10) in 69 cases.

to re-emphasize their belief that patients with an ovarian malignancy in whom only the involved ovary is removed, should have a second operation with removal of the opposite ovary even though it appeared grossly normal during the first operative procedure.

The 3 and 5 year survival rate according to the stage of the disease is shown in Table VIII.

TABLE VIII: THREE AND FIVE YEAR SURVIVAL RATE

Stage	Patients	3 Year survival	5 Year survival
I	21	16 (76.2%)	15 (71.4%)
II	12	7 (58.3%)	6 (50%)
III	32*	10 (31.2%)	8 (25%)
IV	19	5 (26.3%)	4 (21%)
Total	84*	38 (45.2%)	33 (39.2%)

* Three additional Stage III patients not included as complete follow-up was not available.

A five year survival of 39.2 per cent in our series compares favorably with that reported by other authors (5, 7, 10, 11, 16, 17, 18, 20, 27) in patients treated by surgery and postoperative radiation.

When the tumor was confined to the ovaries and completely removed, the five year survival rate was found to be 71.4 per cent. Harris and Payne (3), however, reported an 85 per cent five year cure in 23 patients in whom the entire malignant process was removed without postoperative radiation. These patients would be comparable to our Stages I and II in which our survival rate is only 63.6 per cent. This suggests that it might be better to defer radiation in those patients in whom the tumor is confined to the ovaries and completely removed until there is clinical evidence of possible recurrence. Because of the difficulty in determining whether or not all metastatic disease is removed at operation, we believe patients in Stage II should receive radiation as well as those in Stages III and IV. This treatment must be carried to the maximum allowable depending on the tolerance of the skin and surrounding organs. Where there is evidence of peritoneal transplants the upper abdomen should also be irradiated.

In clinically inoperable patients, many authors (4-6) believe that the results might be improved if preoperative radiation were given before surgery is attempted. Meigs (14) and Pemberton (22) believe that peritoneoscopy might be used in such patients to determine the extent of the disease. If there is evidence of widespread abdominal carcinomatosis, the patient might thereby be saved a needless operative procedure. That radiation will sometimes convert an inoperable tumor into one which is removable surgically has been shown in 3 of our patients and in several cases reported by other authors (1, 21). Parks (21) recently reported 3 patients in which preoperative radiation was effective in reducing the size of the tumor so that it could be removed at operation. In our clinic, as in others (8, 11, 25, 26), it is considered that even in advanced disease an exploration with biopsy is justifiable since it affords an opportunity for more accurate diagnosis and thorough evacuation of abdominal fluid. The small incision is of little more significance than the puncture wound required for paracentesis. The patient should then be given a complete series of radiation treatments followed by a second operation in 6 to 12 weeks with removal of all or as much of the tumor as possible. Further radiation may then be administered as indicated.

The tumor dose necessary to inhibit the further growth of ovarian carcinoma or its secondary transplants has not been established. Healy (4) considers approximately 2 threshold erythema doses adequate to inhibit tumor growth activity in many cases and usually sufficient to cause complete disappearance of all secondary transplants. Montgomery and Farrell (16) consider a minimum of at least 1,600 to 2,000 tissue r an adequate dose. Walter, Bachman, and Harris (27) classified therapy as "adequate" in their group of cases only if a minimum of 1,200 r (in air with backscatter) was delivered to each of 3 pelvic fields in approximately 1 month. For purposes of analyzing results, our cases have been classified into 2 groups: (a) those receiving a small tumor dose, less than 1,500 tissue r (31 patients) and (b) those receiving a larger tumor dose of 1,500 tissue r or more (53 patients). Curves were then made of the survival rates in these two groups of cases as shown in Fig. 2.

A comparison of these curves shows a higher percentage of survivals especially in the first 18 months following treatment and a significantly greater number of 5 year survivals (47.2 per cent) in the group of patients receiving over 1,500 r as compared to a 5 year survival rate of 25.8 per cent in the group receiving less than 1,500 r. This suggests that the difference of 21.4 per cent in the 5 year survival rate of the 2 groups at least in part is due to the effects of radiation.

Since many of the 5 year cures in Stage I group may be due to surgery alone, these patients were omitted and curves of survival again made as shown in Fig. 2.

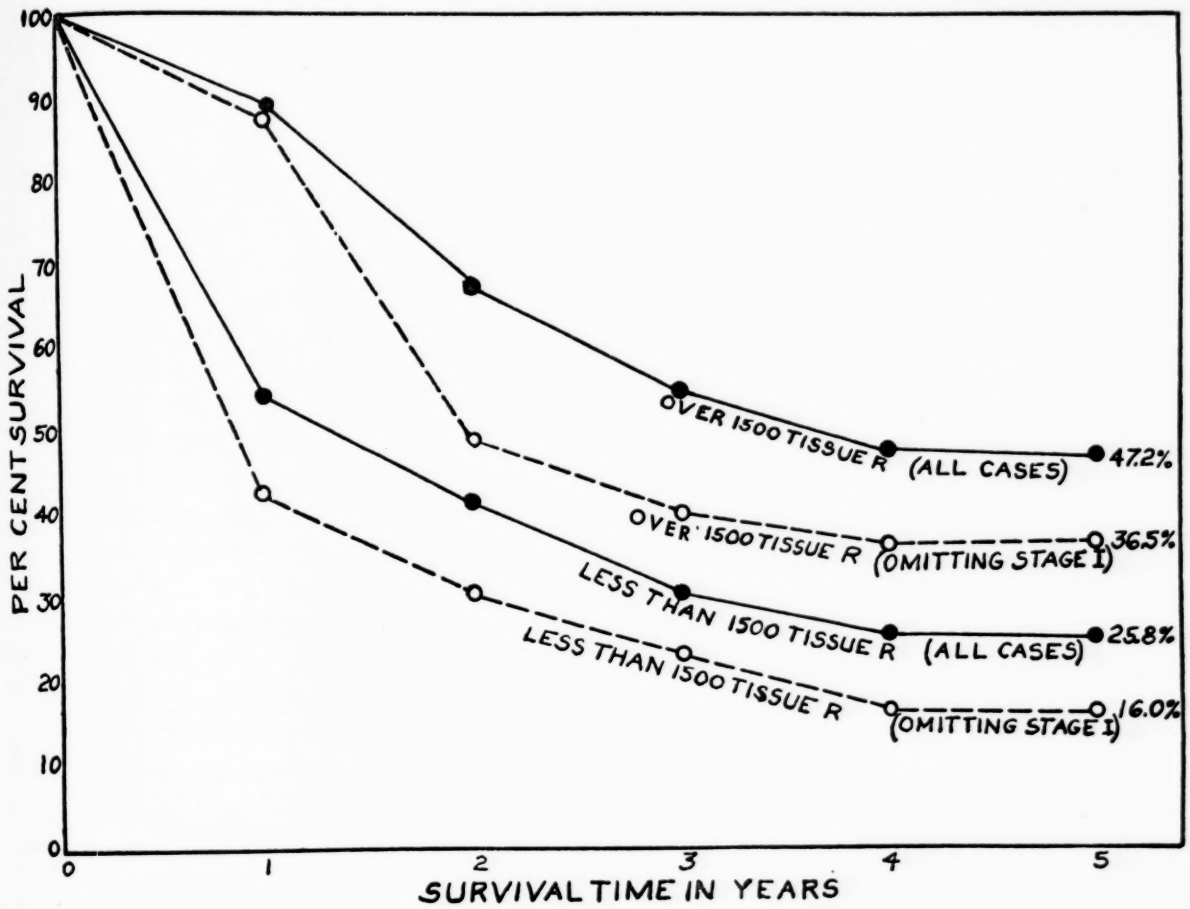

FIG. 2.—Comparison of our survival rate in patients with primary ovarian carcinoma who received a tumor dose of less than 1,500 roentgens with those who received a tumor dose of more than 1,500 roentgens. Solid lines include all cases; broken lines cover all cases except those classified as Stage I.

Of the remaining 63 patients, 25 received less than 1,500 tissue r, while 38 received 1,500 tissue r or more. The 5 year survival rate in the group receiving the larger tumor dose is now 20.5 per cent greater than that for the group given a smaller tumor dose, less than 1,500 tissue r. This again seems to indicate that radiation increased the number of 5 year cures. However, since the percentage difference in the 5 year cure rate is not changed significantly by omitting the patients in Stage I, this would suggest that radiation did not have any real effect on the 5 year cure rate, *i.e.*, in preventing recurrence, in patients whose tumor was localized to the ovaries and completely removed. The small number of patients in our series, however, makes it impossible to draw any definite conclusions.

The histological type did not appear to be of any real value in determining the radiosensitivity or predicting the future growth of the tumor. There was little or no difference in the 5 year survival rate between papillary and non-papillary serous cystadenocarcinoma. Six of the 9 patients with pseudomucinous cyst adenocarcinoma survived 5 years but in 5 of these the tumor was confined to the ovaries and completely removed. There were 3 patients with a carcinoma arising in a dermoid. In 2 of them, the disease had spread beyond the ovary and was incompletely removed. One of these patients survived 6 months, the other, 5½ years. The third patient was living and well 13 years after operation. Two patients with a granulosa cell tumor failed to survive 1 year. A third patient with a granulosa cell tumor is still living and well after 9 years.

The time at which treatment should be started following surgery is a still somewhat unsettled question but many authors feel that treatment should be begun as soon as the patient is able to come to the radiological department, preferably within the first 2 weeks after operation. Therefore, our series has been separated into two groups

—those treated within the first fifteen days (28 patients) and those treated 15 to 70 days after their surgical operation (47 patients). From 3 months to 1 year or more had elapsed following operation before the other 9 patients were referred to the radiological department and therefore they are not included. Curves were made comparing the survival rates in the two groups referred to above as shown in Fig. 3.

A comparison of the survival rates seems to indicate that patients in whom the start of treatment is delayed from 6 weeks to 2 months to give them a chance to recover from their surgical procedure certainly do as well and may perhaps do a little better than those treated within the first 2 weeks. Patients who rapidly fail following their operative procedure are seldom benefited by radiation and may be adversely affected by the added toxemia. The plan in our clinic at the present time is to wait 6 weeks after operation before beginning radiation therapy.

SUMMARY AND CONCLUSIONS

1. The records of 87 patients with a histological diagnosis of ovarian carcinoma who received radiation treatment at the Hospital of the University of Pennsylvania between 1930 to 1941 were reviewed with particular reference to the value of radiation therapy.

2. Seventy-two per cent of the patients were from 40 to 69 years of age.

3. In view of the increased interest in cancer survey studies, the importance of a routine cancer history is suggested.

4. The incidence of sterility (42 per cent) in the married women in our series is high in comparison to a normal incidence of ten per cent. A possible relationship to ovarian cancer susceptibility is discussed.

5. Pain, abdominal swelling, irregularity of menses, or postmenopausal bleeding were the most frequent presenting symptoms. Ascites was present in 34 patients at operation. A pleural effusion

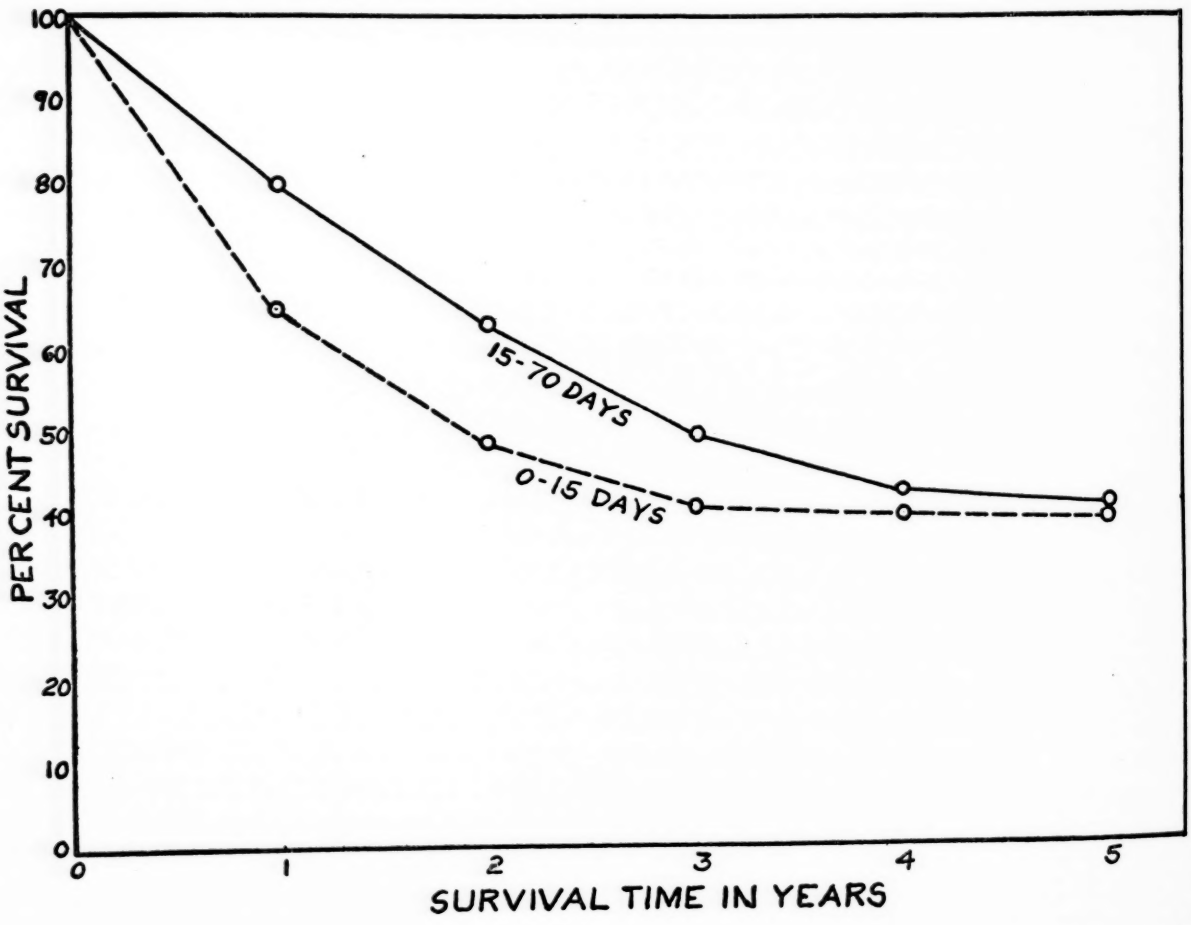

FIG. 3.—Comparison of our survival rate of patients in whom radiation treatment was started within 15 days with those in whom treatment was delayed for 15 to 70 days after their surgical operation.

was noted in 8 patients at sometime during the course of the disease. Since the pleural effusion disappeared after removal of the primary tumor in 2 patients a possible similarity to a Meigs' syndrome is suggested in some of these cases.

6. The clinical stage of the disease appeared to be of greater significance than the histological type in the treatment and prognosis in our series.

7. The total 5 year survival rate in 84 of these patients in whom complete follow-up information was obtained was 39.2 per cent.

8. Adequate surgery is of first importance in the proper management of ovarian carcinoma and should include the removal of both Fallopian tubes, ovaries, and uterus. When only the involved ovary is removed and the lesion proves to be malignant, it is considered advisable in most cases to perform a second operation for removal of the opposite ovary even though it appeared grossly normal.

9. Our results in Stage I indicate that surgery alone gives results comparable to surgery plus postoperative radiation. Therefore, it is believed radiation should be withheld in these patients until there is clinical evidence to suggest a recurrence.

10. Postoperative radiation, however, is of definite value in patients with evidence of extension or metastasis at operation both in the relief of symptoms and prolongation of life.

11. The maximal tumor dose of radiation should be given whenever possible. The increased number of 5 year survivals following tumor doses of 1,500 tissue r or more suggest that radiation has been a significant factor in the results. With smaller quantities of roentgen therapy, the symptoms are occasionally relieved but the life expectancy is seldom improved.

12. Patients with advanced disease should be given the benefit of an exploratory procedure with biopsy and thorough evacuation of abdominal fluid. Radiation should then be given to the maximum tolerance level and followed when possible by a second operation 6 to 12 weeks later. Further radiation may then be administered when indicated.

13. A delay of 6 weeks after operation before starting roentgen therapy is advocated to give the patient a better chance to recover from the surgical procedure.

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Lack of Extractable Carcinogens in the Skin of Patients With Multiple Precancerous Keratoses of Actinic Origin

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Although it is well known that ultraviolet light exerts a potent carcinogenic effect upon the skin, it is not known whether this effect is exerted directly upon a chromosome or a gene, or whether it is exerted indirectly through the formation of a carcinogenic compound. Indirect evidence that ultraviolet light does not exert its carcinogenic effect through the formation of a chemical resembling the carcinogenic hydrocarbons was provided by the observations of Rusch, Kline and Baumann (8) when they showed that in contrast to the additive effect between different carcinogenic hydrocarbons there was no additive effect between ultraviolet rays and carcinogenic hydrocarbons. Unpublished experiments by these same authors giving direct evidence of the lack of formation of carcinogenic chemicals by ultraviolet irradiation showed that benzene extracts of irradiated mouse skin or of irradiation tumors failed to induce neoplasms when injected subcutaneously in corn oil. Similarly, Passey (6) was unable to demonstrate carcinogens in extracts of the skins of rats irradiated for 1 year with ultraviolet rays. The observations of these workers were made with relatively small amounts of material so there was a possibility of insufficient dosage.

The possibility that the skin of patients with multiple precancerous keratoses of actinic origin might contain extractable carcinogenic chemicals was considered. The availability of relatively large areas of keratotic skin for extraction by washing with benzene offered the advantage of providing large amounts of material for exhaustive tests for possible carcinogenic activity. Furthermore, by repeatedly washing one side of the face with benzene and observing the incidence of carcinoma on that side as compared to the unwashed side it was reasoned that, if carcinogens were being formed, their presence would be reflected in a reduced incidence of cancerous change in the lesions on the washed side of the face.

MATERIALS AND METHODS

Selection of patients.—Twelve patients with multiple precancerous keratoses of the face were selected. All of these patients had previously developed at least one carcinoma and most had developed several carcinomas in their keratoses. Except for one 9 year old boy with xeroderma pigmentosum and one young woman with delayed xeroderma pigmentosum, the patients were elderly people with typical multiple actinic keratoses or the so-called "farmers' skin" or "sailors' skin".

The patients were given a supply of 2 × 2 inch gauze pads and a supply of benzene. They were instructed to wash one side of their faces with the benzene and to save the pads used for this purpose in a large bottle. The procedure was repeated daily for periods of from 20 to 706 days with an average period of 162 days.

In addition, small amounts of skin washings and excised keratoses from 25 other patients with multiple keratoses were pooled for extraction with benzene and assay for carcinogenic activity.

In most cases, a record was kept of the size and location of the keratoses on both sides of the face so that any change in size or development could be followed.

Preparation of the extract.—The pads containing the skin washings were continuously extracted with hot benzene in a Soxhlet extractor for a minimum of 8 hours. The benzene extract was then concentrated by heating over a water bath under vacuum. The final product was a thick yellow or brown oil which became semisolid in the refrigerator.

Tests for carcinogenicity.—The concentrated material made from the skin washings was tested for carcinogenicity in mice by the following means: (a) painting on the skin in benzene solution (b) painting on the skin in combination with croton oil (c) painting on the ear plus irradiation with ultraviolet light, and (d) subcutaneous injection in mice and rats; in addition whole keratoses were implanted subcutaneously.

RESULTS

Painting on the skin.—The material extracted from precancerous skin was painted on the skin of mice in two experiments.

In the first experiment, the material was dissolved in benzene so that 1 cc. of the benzene extract contained the amount of material washed from the skin in an average period of 1.7 days. Ten white mice derived from the Bar Harbor A.B.C. stock were used and the material was painted on the interscapular region after clipping the hair. Three strokes with a camel's hair brush were made 3 times a week. This was continued until all the mice were dead. Four mice were still alive at the end of 1 year of painting and the last mouse died after 22 months. There were no developments suggestive of precancerous or cancerous changes in any of the painted mice. There was some scaliness and some epilation in a few of them but no more than would be expected from the benzene alone. No carcinomas were found elsewhere in the bodies of these mice.

The second experiment was carried out mainly for the purpose of determining if an increased dosage of the extract might result in the production of neoplasms. Therefore in this experiment, the dosage was increased so that 1 cc. of the benzene solution contained the material from 3 daily washings of the skin of the patients with precancerous lesions. For control purposes another group of mice were similarly painted with a benzene solution of 0.2 per cent of methylcholanthrene. Twenty mice were used in each group. After 6 months of painting 3 times a week, the mice painted with the skin extract showed no precancerous or cancerous changes whereas 100 per cent of the 14 remaining mice in the group which were treated with methylcholanthrene had developed carcinoma. The painting with the skin extract was continued and at the end of a year there still was no neoplastic change in the eight surviving mice. The last mouse died after two years of painting and in no instance did carcinoma develop. The only neoplasm to appear in the group was a lymphosarcoma of the spleen.

Painting on the skin in combination with croton oil.—From the group of mice remaining after a year of painting with the skin extract in the preceding experiment, 5 were selected for painting with croton oil in combination with the skin extract. The croton oil was added to the benzene solution of the extract in a concentration of 0.5 per cent.

The purpose of the croton oil, a co-carcinogen described by Berenblum (1) was to produce an inflammatory reaction which might elicit frank carcinoma from a possible latent or preneoplastic stage. Continuation of the painting for a period of 1 year, when the last mouse died, resulted in the formation of no carcinomas although there was considerable epilation, thickening of the skin, and some ulceration and crust formation. After 5 months of this combined painting there were 5 mice living, and at 9 months 2 survived.

Painting on the ear plus irradiation with ultraviolet light.—To determine whether the material extracted from precancerous human skin might have an augmenting effect on carcinogenesis by ultraviolet light, the right ears of 24 A.B.C. mice were painted with the material before irradiation, while the left ears were left unpainted. Ultraviolet irradiation was carried out three times a week in wire holders according to the technic of Rusch and his associates (7). In addition 25 mice which were not irradiated were also painted three times a week on the right ear with a benzene solution, 1 cc. of which contained the material from 1.7 daily washings.

Inasmuch as the time of onset of the carcinomas was essentially the same in the painted and unpainted ears no detailed tabulation of the results is given. By the fourth month the ears became thickened and nodules began to appear; by the eighth month 10 mice were left in the irradiated group and all had cancers affecting both ears about equally; after 1 year all had died of cancer. The neoplasms were sarcomas and squamous cell carcinomas and the incidence of the two types was similar in both ears.

No cancers were produced in the non-irradiated mice though the right ears were painted thrice weekly till the last mouse died. Nineteen mice were alive at 8 months, 4 were alive at 16 months and the last mouse died after 20 months. In no instance was any abnormality noted on the ears other than the occasional hyperkeratosis due to the benzene.

Therefore, the results showed no augmenting effect of the skin extracts upon ultraviolet carcinogenesis and the skin extracts by themselves again failed to induce cancer.

Subcutaneous injection in mice and rats.—The sebum extracted from the precancerous skins was injected in single doses into 20 mice and into 10 rats. The material was also injected in multiple doses into 30 mice. One cubic centimeter of the sebum represented the amount of material obtained

from the skins of patients by daily washing for periods of from 22 to 65 days.

In a preliminary experiment one mouse received an injection of 0.25 cc. of the thick yellow oil while the second mouse received 0.35 cc. It was observed that this dosage was too great inasmuch as it led to the development of sterile abcesses and ulcers. Accordingly, the dosage was reduced to 0.1 cc. in a group of 8 mice and 0.05 cc. in 0.05 cc. of corn oil in a group of 10 mice. In addition 10 rats each received an injection of 0.15 cc. of the undiluted extract.

These single injections resulted in the formation of nodules that persisted for many months but in no instance did a neoplasm develop at the injection site. After 1 year there were 16 mice and 4 rats living. After 2 years only 2 mice and 2 rats were still alive. Several subcutaneous nodules, which had persisted for periods varying from 14 to 26 months, were studied microscopically and were found to consist of masses of amorphous material surrounded by dense fibroblastic proliferation. In no instance was neoplasm present. Two adenocarcinomas arose in the mammary glands and one sarcoma appeared on the foot of a mouse, but since none of these neoplasms were in contact with injected material, they were assumed to be of spontaneous origin.

In 30 mice repeated injections of 0.1 cc. of the extract were given at intervals of 1 month. Eleven mice received 2 injections, 5 received 3, 10 received 4, and 4 received 7 injections. An attempt was made to re-inject into the original injection site in the interscapular region. Most of the mice developed persistent subcutaneous lumps and a few developed ulcers, but in no instance did a malignant neoplasm develop at the site of injection. The mice were kept until they died when they were examined to determine the cause of death. After a year 18 of the 30 mice were still living. After 18 months there are 4 mice living and none of these have any evidence of neoplastic change in the injection site. Only 1 neoplasm was observed—a fibrosarcoma which arose in the mammary gland at some distance from the injection site.

Accordingly, it is concluded that the sebum extracted from the skin of patients with multiple precancerous lesions contained no carcinogenic substances which could be demonstrated by subcutaneous injections in mice and rats.

Implantation of whole keratoses.—Senile keratoses excised from 9 different patients were implanted in the subcutaneous tissues of 20 Sprague-Dawley rats. After one year all 20 of the mice were still alive and many of them had nodules, but in none did cancer develop. After 18 months no neoplasms had developed in the 8 rats remaining; therefore, they were discarded. It is concluded that subcutaneous implantation of keratoses does not elicit neoplasia.

Effect of washing the precancerous skin with benzene on the incidence of cancer.—The patients were requested to wash only one side of their face with the benzene and to leave the other side unwashed as a control. The keratoses on both sides of the face were measured with calipers at each visit. When carcinoma developed, the lesions were excised by the chemosurgical technic (5).

Washing had but little effect on the keratoses; most of the lesions continued to enlarge, others regressed somewhat, but the same thing occurred on the unwashed side. One senile keratosis was observed to regress completely after vigorous washing with benzene. The patient stated that he felt stinging sensation in the lesion and it seems likely that the tissues were so defatted by the benzene that some of them were killed with resultant eradication of the keratosis.

The incidence of cancer in the 12 patients with multiple precancerous lesions was the same on both the washed and unwashed sides of the face. On the washed side there developed 3 squamous cell and 4 basal cell carcinomas while on the unwashed side there developed 2 squamous cell and 5 basal cell carcinomas. Therefore, washing the precancerous skin with benzene has essentially no effect either on the growth of the keratoses or on their tendency to become cancerous.

DISCUSSION

Although the foregoing experiments show a complete lack of carcinogenicity in the material washed with benzene from the skins of patients with multiple precancerous lesions, there remains the possibility that carcinogens might be extracted by other means. Benzene was chosen as the solvent because practically all of the substances known to be carcinogenic for the skin are soluble in it. However, although it seems improbable, it is conceivable that carcinogenesis by ultraviolet rays may be accomplished by the production of a water-soluble carcinogen.

It is also conceivable that the sought-for carcinogen might be so firmly bound to some cell constituent, possibly a protein, that it would not be obtained by simply washing the skin with benzene. Even if the carcinogen were not so bound, it is possible that the hypothetical carcinogen might be formed and retained in the basal cell layer from

which it might not be extractable by washing the surface of the skin. However, since sebum and desquamating horny scales are derived from basal cells this seems unlikely.

The possibility that the sebum itself might mask or inhibit the action of some carcinogenic substance is suggested by the findings of Simpson, Carruthers, and Cramer (10) who reported an inhibitory effect of lanolin upon methylcholanthrene carcinogenesis. However, Kline (7) has shown that the inhibitory effect of lanolin is lost when the methylcholanthrene and lanolin were dissolved in benzene and painted on the skin of mice. Therefore, since the material from precancerous skin was applied to the mice in benzene solution the sebum probably had no inhibitory effect on any carcinogenesis that might have taken place. Furthermore, Kline (4) has observed that carcinogenesis by benzpyrene is not inhibited by the use of lanolin as a vehicle for subcutaneous injection. Therefore, it seems unlikely that the sebum in the human skin extract would inhibit any carcinogen that might be present in the injected material. Further experiments to elucidate this point are in progress, however.

The lack of augmentation of ultraviolet carcinogenesis by the application just before irradiation of a benzene solution of the material from precancerous skin is evidence that this human sebum contained no special substance that was not already present, in the mouse at at least upon which the rays might act to produce carcinogens.

One reason for persistently trying to demonstrate carcinogens in material washed from human skin was the well-known relation between non-circumcision and concer of the penis which apparently is caused by the action of the retained smegma. A similar situation is the occasional origin of cancer in the lining of sebaceous cysts; in this case the sebaceous material appears to be the carcinogenic substance. If the materials thrown off from the skin and its appendages can act as carcinogens under such circumstances, why do the washings from precancerous skin fail to exhibit carcinogenic properties? One possible answer is that the rancidity which characterizes retained smegma and the contents of sebaceous cysts may be essential to the carcinogenicity of these materials.

Some success in the search for endogenous carcinogens has been attained by Schabad (9), Steiner (11), Hieger (3) and des Ligneris (2) but these workers usually used liver as the source of tissue. Steiner extracted burn and x-ray scars and found no carcinogenic activity, but he did observe 1 sarcoma in the region injected with material extracted from an epidermoid or sebaceous cyst (12).

In the absence of demonstrable carcinogens the material extracted from precancerous skin was not subjected to spectroscopic or other analysis for chemicals similar to the carcinogenic hydrocarbons. However, the material was shown to have an intense, bluish fluorescence in ultraviolet light not unlike the fluorescence of keratoses.

The failure of washing with benzene to reduce the incidence of carcinoma in the skin of patients with multiple precancerous lesions is consistent with the failure to find carcinogens in the material washed from that type of skin.

SUMMARY AND CONCLUSIONS

Material washed with benzene from human skin affected with multiple precancerous keratoses contained no carcinogenic substances that could be demonstrated either by painting on the skin of mice or by injection into the subcutaneous tissue of mice or rats. Furthermore the material failed to augment ultraviolet carcinogenesis in mice when painted on the skin just before irradiation and it failed to produce precancerous changes from which carcinoma could be elicited with croton oil. Implantation of senile keratoses into rats also failed to demonstrate carcinogenic substances in these lesions.

Repeated washing of precancerous skin with benzene to remove hypothetical carcinogens did not reduce the incidence of cancerous change in the keratoses.

These findings are believed to be evidence in favor of the hypothesis that the carcinogenic influence of ultraviolet rays is exerted by a direct effect upon the cell constituent responsible for neoplastic change rather than by an indirect effect through the formation of a carcinogenic chemical similar to the carcinogenic hydrocarbons.

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The Effect of Carcinogenic Compounds on the Ascorbic Acid Content of the Liver in Mice and Rats

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The experiments recorded below are a continuation of an earlier investigation (8) on the effect of aromatic compounds, carcinogenic and non-carcinogenic, upon the ascorbic acid content of the liver in mice.

In the present series, four compounds were used, namely *p*-dimethylamino-azobenzene (*p*-DA), 2-acetamidofluorene (AAF), 7-hydroxy-2-acetamidofluorene (7-OH-AAF) (4) and azobenzene (AB). The experiments fall into two groups: (a) *Short term (up to 13 days) injection experiments.* Subcutaneous and intraperitoneal injections in mice (CBA) and intraperitoneal injections in rats (Wistar and black-and-white) of AAF and 7-OH-AAF only. (b) *Long-term (up to 460 days) feeding experiments* which give time for the development of tumors. The four compounds were added to various diets given to Wistar rats which were used in other investigations as well (3, 5).

METHODS

The liver was removed at once, and the whole organ, in the case of a mouse, or one or more portions of similar weight (about 1 gm.) from the liver of a rat, homogenized by the method of Potter and Elvehjem (9) in 10 ml. 3 per cent metaphosphoric acid, and centrifuged, and the process of mixture and centrifugation repeated twice, using 30 ml. metaphosphoric acid in all. The extract was titrated with 2.6-dichlorophenolindophenol. The substance in mouse liver which reacts with this dye, and the increment of it induced by carcinogens, is destroyed by vegetable marrow juice (8) and may therefore be assumed to be ascorbic acid.

I. Injection Experiments on Mice and Rats

The animals used in these experiments received the ordinary mixed diet given to the laboratory stock. The water content of the livers was not estimated.

(a) *Mice.*—Fifty-two mice (CBA males and females) received 2 intraperitoneal or subcutaneous injections of AAF or 7-OH-AAF (10 mgm. in 0.2 ml. arachis oil) or of 0.2 ml. of arachis oil only over a period of 4 to 6 days; they were killed between the sixth and 13th day after the first injection (Table I). Controls receiving arachis oil are essential because this oil, and sesame oil, alone, increase the ratio of liver weight to body weight in male mice, *e.g.* from 4.8 to 5.4 per cent (7). The figures for the concentration of ascorbic acid in the liver show the wide range of variation found in mice (7), but there is in the male CBA mice a statistically significant increase of 23 per cent following injection of 2-acetamidofluorene, while the females show no significant change. The ratio of liver weight to body weight shows no distinct differences in the various series. Intraperitoneal injection of 7-OH-AAF produced no increase in the ascorbic acid in either sex.

(b) *Rats.*—Sixty-three rats (Black-and-White, Wistar, males and females) received 2 intraperitoneal injections for from 3 to 5 days of AAF or 7-OH-AAF (30 mgm. in 1 ml. of arachis oil) or of 1 ml. arachis oil only: they were killed between the fifth and eighth day after the first injection (Table II).

The black-and-white rats showed a mean increase in concentration of ascorbic acid in the liver after injection of AAF which was of the same degree (34 to 35 per cent) in both sexes, and was therefore greater than that seen in the CBA mice. The 7-OH compound produced only a very slight increase.

Data on the Wistar rats reveal a very wide range of variation. Injection of AAF produced a mean increase in both sexes of only about 10 per cent, which is no greater than that seen in the black-and-white rats after injection of the 7-OH compound, whereas the mean figures obtained in 4 rats with this latter compound were practically the same as those from the controls, 269 and 267 in males; 232 and 235 in females.

The ratio of liver weight to body weight shows no constant differences in the various series. Hence the results of the injection experiments show: (a) that the black-and-white rats respond to the action of AAF by an increased concentration of ascorbic acid in the liver much more than do Wistar rats;

TABLE I: INJECTION EXPERIMENTS IN CBA MICE WITH 2-ACETAMIDOFLUORENE

Experiment	Day of injection	Killed on day after first injection	Ascorbic acid in liver, μgm. per gm. wet weight: Male Control S.	Male Control P.	Male A.A.F. S.	Male A.A.F. P.	Male 7-OH A.A.F. P.	Female Control S.	Female Control P.	Female A.A.F. S.	Female A.A.F. P.	Female 7-OH A.A.F. P.
1	1,5	6	...	289	385	351		266	...	297	352	
		12	322	...	428	469		...	345	272	262	
	1,4	7	...	361	...	388		...				
2		9	348	...	394	...		...				
		10	...	296	...	370		...				
		13	351	...	...	...		298				
								351				
3	1,6	8	270	...	...	373		...	238	...	258	
		9	307	...	...	335		...	272	...	290	
4	1,5	6	...	226	...	370	260	...	292	...	351	278
5	1,6	6	...	226	...	301	220	...	217	...	315	226
		11	...	362	...	380	365	...	272	...	330	310
		Mean	340	297	402*	371†	283	305	284	299	304	271
			309		379			290		303		
			100		123			100		104		

*P 0.02 S = Subcutaneous injection
†P 0.001 P = Peritoneal injection.

(b) that the sexual difference in CBA mice, in that the males respond to AAF and the females do not, is absent in rats; and (c) that 7-OH-AAF is almost or quite inactive both in rats and in male mice.

II. Feeding Experiments on Wistar Rats

A semi-synthetic diet with and without the addition of *p*-DA, AAF or 7-OH-AAF was given, as follows:

		Calories, %
Starch	1,260	39.6
Sugar	630	18.6
Casein (fat & vitamin free)	342	12.7
Casein (commercial)	177	
Lard	420	29.1
Cod liver oil	7	...
Mineral salt mixture (Glaxo)	126	...

The diet was supplemented daily with 1 or 2 gm. of carrots or greens and about 1 gm. of "National Bread" (80 to 82 per cent extraction). The AAF, *p*-DA or 7-OH-AAF was added in amounts to give a final concentration of 0.07 per cent. Growth was about the same on the control diet and on those containing *p*-DA, AAF and 7-OH-AAF.

Another diet of rice with and without the addition of 6 per cent casein and of *p*-DA or AB (0.06 per cent) was tried. The rats, which weighed about 100 gm. at the beginning of the experiment, ceased to grow on these diets.

The concentration of ascorbic acid is expressed per gm. dry weight, as the water content of the livers of animals on the semi-synthetic diet receiving carcinogenic compounds, and that of the tu-

TABLE II: INJECTION EXPERIMENTS IN RATS, WITH 2-ACETAMIDOFLUORENE

Experiment	Day of injection	Killed on day after first injection	Ascorbic acid in liver, μgm. per gm. wet weight: Male Control	Male A.A.F.	Male 7-OH—A.A.F.	Female Control	Female A.A.F.	Female 7-OH—A.A.F.
			BLACK-AND-WHITE					
1	1,3	5	527	418	...	234	297	...
2	1,5	6	294	355	...	275	330	...
		8	296	319	...	244	200	...
3	1,4	5	268	398	385	223	364	240
		8	201	392	246	235	345	226
4	1,4	5	274	350	310	228	385	272
		8	226	319	290	228	318	284
		Mean	270	364	308	238	320	255
			100	135	114	100	134	107
			WISTAR					
1	1,4	5	176	266	...	249	178	...
		8	332	425	...	259	273	...
2	1,4	5	310	277	...	233	288	...
		8	362	195	...	240	295	...
3	1,4	5	204	320	276	179	259	205
			228	...	258	225	293	266
		Mean	269	297	267	232	263	235
			100	110	...	100	113	...

TABLE III: ASCORBIC ACID CONTENT OF LIVERS FROM WISTAR RATS ON SEMI-SYNTHETIC AND RICE DIETS

Compound added	Sex	Number of rats	Liver weight per cent of body weight Mean	Total solids per cent Mean	Ascorbic acid: μgm./gm. dry weight of liver Range	Mean	P.	Ascorbic acid: μgm./gm. body weight Range	Mean	P.
SEMI SYNTHETIC DIET										
None	♀	6	3.5	32.1	675– 870	754	....	7.0– 9.2	7.8	....
	♂	4	3.8	32.4	695– 836	782	....	6.5– 9.2	8.3	....
7-OH-2Acet-amidofluorene.	♀	6	3.7	32.2	628– 995	796	0.5 –0.6	7.3–11.8	9.6	0.05–0.02
	♂	6	4.4	32.9	555–1005	801	0.8 –0.7	9.1–13.5	11.3	0.01
2-Acet-amidofluorene.	♀	7A	5.0	29.2	655–1441	885	0.02	10.3–16.9	13.3	<0.01
	♀	4B	6.5	29.8	675–1290	956	<0.01	13.3–19.8	17.1	....
	♀	2C	..	25.6	580–1190	885	....	....	....	....
	♂	5A	5.8	27.2	513–1320	998	<0.01	6.9–24.2	15.4	<0.01
	♂	4B	6.2	25.9	698–1270	890	0.1 –0.05	12.2–19.0	14.9	....
	♂	1C	..	27.5	....	455	....	....	..	....
p-Dimethylamino-azobenzene	♀	8B	4.3	27.4	565–1280	947	0.05–0.02	13.3–32.0	21.9	<0.01
	♀	6C	..	20.5	495– 910	697	....	....	..	....
	♂	2A	5.8	28.2	1118–1330	1224	<0.01	10.2–25.0	17.6	....
	♂	7B	5.0	27.4	730–1390	941	0.2 –0.1	11.4–22.7	18.1	0.01
	♂	6C	..	20.3	380–1340	940	....	....	..	....
RICE DIET										
p-Dimethylamino-azobenzene	♀	8	3.4	29.5	463– 930	649	....	4.3– 9.9	7.0	....
	♀	3B	5.2	27.8	710– 770	730	0.2 –0.3	10.8–10.9	10.1	0.02–0.01
Azobenzene	♀	7	4.4	31.0	840–1310	933	<0.01	8.5–16.7	11.8	<0.01
p-Dimethyl-aminoazo-benzene	Rice+ 6% casein ♀	7	3.5	30.6	677– 786	739	....	5.5–11.0	7.8	....
		8A	4.8	29.7	740–1010	864	0.05–0.1	10.4–22.7	14.3	<0.01
		12B	4.9	29.5	670–1170	863	0.05–0.1	9.4–16.1	11.9	<0.01
		1C	..	19.8	....	900	....	....	..	....

A—Livers without tumors. B—Normal parts of livers bearing tumors. C—Tumor tissue.

mors, was generally higher than that of the normal liver. In view of the increase in liver weight in the treated animals, the ascorbic acid content of the liver is expressed as μgm. per gm. of body weight, and not as the whole content of the liver.

RATS ON A SEMISYNTHETIC DIET. (TABLE III)

The water content of the liver was increased by *p*-DA and AAF, but not by 7-OH-AAF. In the group receiving AAF the ascorbic acid concentration (dry weight) is increased significantly in both sexes, as in those animals receiving *p*-DA, except in tumor-bearing males, where the result is perhaps affected by the presence of necrotic tissue.

When these data were calculated on a wet weight basis, no significant increase was found in either group. The figures are therefore omitted from the table.

Rats receiving 7-OH-AAF show no appreciable variation from the control group on either a dry or a wet weight basis.

The ascorbic acid content of the liver (μgm./gm. body weight) showed a striking increase among all animals receiving *p*-DA and AAF and a slight increase in those receiving 7-OH-AAF.

A concentration of ascorbic acid (dry weight) as high as that in the liver appeared in some of the tumors.

RATS ON RICE DIET. (TABLE III)

The concentration of ascorbic acid was greatly increased (wet and dry weight) by AB; was not increased by *p*-DA (wet weight) and only but barely so in some of the series on the dry weight basis.

The ascorbic acid content of the livers, calculated as μgm. per gm. of body weight, was raised significantly by AB, and by *p*-DA (up to 100 per cent in one series). The water content of the liver was not altered.

DISCUSSION

Differences in results corresponding to differences in species, strain, sex and method of administration make generalization difficult in this subject as in other branches of cancer research. The data now available are summarized in Table IV. In earlier short-term (2 to 19 days) experiments (8) injection of seven carcinogens increased the concentration of ascorbic acid in mice; the very slightly carcinogenic 1,2-benzanthracene was equally active, while the three non-carcinogenic compounds (anthracene, phenanthrene, naphthalene) gave negative results. The investigation has now been extended to include short-term injection and long-term feeding experiments on rats. No short-term injection experiment comparable to those described

above was made with AB, which so far as we know is non-carcinogenic, but the ascorbic acid was increased by long-term feeding with this compound. *p*-DA gave a more definite positive result when fed to rats than when injected in mice (8). A very distinct change is produced in AAF by the insertion of the 7-OH group, giving a compound which is much less carcinogenic (Hoch-Ligeti, unpublished experiments) and in which the power to increase the ascorbic acid in the liver is much diminished. The greater response to the injection of AAF in black-and-white in contrast to Wistar rats (Table II) is perhaps of interest in view of the increased susceptibility of the latter to the induction of mammary tumors (1).

TABLE IV: SUMMARY OF RESULTS

Compound	Method of administration	Species and strain	Ascorbic acid in liver*
3,4-Benzpyrene	Injection subcut. and intraperit.	CBA ♂ mice	+
Cholanthrene	Injection intraperit.	" " MRC ♀ "	+
Methyl cholanthrene	" "	" " CBA ♀ "	+
9,10-Dimethyl-1:2- Benzanthracene	" "	CBA ♀ "	+
1,2,5,6-Dibenzanthracene	" "	MRC ♂ "	+
1,2,5,6-Dibenzphenanthrene	" "	" " "	+
Stilbestrol	" "	" " "	+
1,2-Benzanthracene	" "	CBA ♂ "	+
2-Acetamidofluorene	" subcut. and intraperit.	" " "	+
"	" " "	" ♀ "	−
"	" intraperit.	Blace & White rats ♂ and ♀	+
"	" "	Wistar rats ♂ and ♀	+(slight)
"	Fed.	" " " " "	+
p-Dimethylaminoazobenzene	Injection intraperit.	CBA ♂ and ♀ mice	±
"	Fed.	Wistar rats ♂ and ♀	+
2,2′-Azonaphthalene	Injection intraperit.	Stock ♂ mice	+
Azobenzene	Fed.	Wistar ♀ rats	+
9,10-Dimethylanthracene	Injection intraperit.	MRC ♀ mice	−
Anthracene	" "	CBA ♀ "	−
Phenanthrene	" "	MRC ♂ "	−
Naphthalene	" "	" " "	−
7-OH-Acetamidofluorene	" "	CBA ♂ and ♀	−
"	" "	Black & White rats ♂ and ♀	+(very slight)
"	" "	Wistar rats ♂ and ♀	−
"	Fed.	" " " " "	−

* + indicates an increase, − no increase, ± an inconclusive result.

From the values for ascorbic acid, and for water content of the liver (Table III), it would appear that in animals on a semi-synthetic diet, in which hepatic tumor development is rapid, a definite increase in ascorbic acid is accompanied by a rise in the water content of the liver tissue, whereas animals on a rice diet that develop tumors slowly show a barely significant rise in ascorbic acid and no change in water content. The result of the short-term experiments (Tables I and II) indicate that the increase in ascorbic acid precedes any rise in water content. Hence it would seem that increases in ascorbic acid and water content of the liver are related to the rate of tumor growth, although it is possible that all three effects are due to nutritional causes.

The level of ascorbic acid following administration of carcinogenic substances rose sharply after injection in black-and-white rats (Table II), and after feeding in Wistar rats, and reached a value at which it remained raised. As the ascorbic acid content of the cells in embryonic tissue is increased during active histogenesis (ref. 2, page 136—$AgNO_3$ method) changes in the liver produced by carcinogenic compounds may resemble those of differentiation from one tissue into another. Support for the supposition that changes in the differentiation process of liver cells occur soon after feeding carcinogens might be drawn from the fact that the withdrawal of *p*-DA from the diet after about 50 days does not arrest the development of tumors.

An alternative explanation for the increase in ascorbic acid might be drawn from consideration of cell metabolism. During increased metabolic processes ascorbic acid accumulates in the Golgi apparatus of the cell according to Bourne (2, page 36). The rise in the ascorbic acid content of the liver tissue on administration of carcinogens might be, therefore, an expression of increased metabolic activity, possibly connected with detoxication of the carcinogen. This suggestion might account also for the increase in ascorbic acid content observed on feeding azobenzene. This compound is not car-

cinogenic but nevertheless may provoke similar detoxification processes.

SUMMARY

The effect of carcinogens on the ascorbic acid content of the liver has been further investigated. 2-Acetamidofluorene increased the ascorbic acid content of the liver in CBA male mice, in black-and-white rats, and to a less extent in Wistar rats, when injected, and in Wistar rats when fed; 7-OH-2-acetamidofluorene was much less active.

The ascorbic acid content was increased when *p*-dimethylaminoazobenzene and azobenzene were fed to Wistar rats.

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A.A.A.S. Chemical Research Conference on Cancer, 1947

Abstracts of Papers Presented

METABOLIC AND ENZYMATIC CHARACTERISTICS OF LYMPHOSARCOMA. W. H. SUMMERSON, H. GILDER, and J. M. LEE. (Department of Biochemistry, Cornell University Medical College, New York 21, N. Y.)

The Gardner mouse lymphosarcoma was used in these studies in the form of washed suspensions of discrete viable tumor cells. The aerobic respiratory and glycolytic metabolic characteristics of the cells were measured in bicarbonate-containing media, over the pH range of 6.9 to 7.6, using the Summerson differential manometer. Parallel measurements of glucose utilization and lactic acid production, as determined by chemical analysis, were also carried out.

Oxygen consumption values, expressed as microliters per 10^6 cells per hour, ranged from 0.86 at pH 6.9 to 0.77 at pH 7.6, with a value of 0.80 at pH 7.4. Since 10^6 cells have a dry weight of approximately 0.15 mgm., oxygen consumption per mgm. dry weight per hour (the conventional basis) is approximately 5.0 microliters at pH 7.4.

The respiratory quotient (R.Q.) varied from 0.89 at pH 6.9 to 0.78 at pH 7.6, averaging 0.83 at pH 7.4. Changes in R.Q. with change in pH were therefore slight and paralleled changes in oxygen consumption.

Aerobic glycolysis values were markedly influenced by pH, ranging from 0.30 microliters of acid per 10^6 cells per hour at pH 6.9 to 1.05 microliters at pH 7.6; at pH 7.4 a value of 0.78 was obtained. Furthermore, aerobic glycolytic rate proved to be a *linear function of pH* over the range studied. This marked variation in glycolytic rate with change in pH, unaccompanied by an equivalent variation in oxygen consumption, makes it appear unlikely that there is any direct connection between oxidative and aerobic glycolytic processes in the tissue studied.

Further studies showed that the only acid produced aerobically by the lymphosarcoma cell is lactic acid, and that when glucose disappears from the medium, substantially an equivalent amount of lactic acid results. This indicates that exogenous glucose is utilized aerobically by the cell primarily, if not entirely, for glycolytic processes and not for oxidative purposes. The nature of the substrate being oxidized under these conditions is unknown, but it is not endogenous carbohydrate. This problem is being further investigated.

STUDIES IN SURVIVAL. M. B. MELROY, and R. R. SPENCER. (National Cancer Institute, Bethesda 14, Maryland)

During the past 8 years, studies have been made of the action of certain carcinogenic and other agents upon free-living, single-cell species, based upon the assumption that the process we call carcinogenesis and the process by which such organisms become adapted to unfavorable environments may have certain features in common.

Essentially three concepts are involved in this theory:

1. That the cancer process is a special type of cellular adaptation to various unusual environments. Not all cell adaptations end in cancer. Reptiles and amphibians rarely respond to carcinogenic agents. Cancers may now be induced in animals susceptible to the cancer process in a number of ways.

2. That the transformation of a normal cell to a cancer cell involves a step-like process. There is no clinical or experimental evidence to support the assumption that the genesis of a cancer cell involves a sudden change in a normal cell. On the other hand, the clinically observed association of cancer with chronic irritation; the various precancerous lesions and stages; the prolonged incubation period in all experimentally induced cancers; and such fundamental observations as Earle's transformation *in vitro* of a single clump of normal cells to cancers of 6 or 7 different types of behavior, all seem to fit in with the concept of an adaptive gradualism in the genesis of cancer.

3. That the cancer process is basically a survival mechanism. It has been pointed out that the universal goal of living things is survival, but the clinical and experimental evidence suggests that the survival value of the cancer cell is a thing apart from the survival of the organism as a whole.

The successful immunization of individual animals to multiple lethal doses of various toxins is well known, and can be achieved best by means of interval injections of increasing doses over a considerable period. Naturally, one suspects that the permanent and successful adaptation of a race or species, in contrast to the adjustment of the individual cell or organism, would require a much longer time. However, it is believed that the underlying biochemical mechanism of species adaptation must, of necessity, be basically similar to the mechanism of adjustment of an individual organism.

A cancer cell is conceived of as a descendent of a normal cell that has gradually adjusted successfully over many cell division cycles or generations to an unfavorable environment. The adjustment is successful from the frame of reference of the cell. It is unsuccessful from the frame of reference of the organism as a whole, since the cancer cell becomes parasitic upon its host.

Our studies on bacteria and paramecia have yielded results which have suggested that there are several important principles involved in the mechanism by which

cells become adapted to unfavorable environment. These are:

1. The continuous exposure of actively multiplying, free-living, species to an unfavorable environment may not be fatal to individual organisms or cultures for a number of generations or cell-division cycles, but in due time the species will die.
2. By discontinuous or alternating exposure, an actively multiplying species can adapt and continue to survive in an environment that is fatal when the exposure is continuous.
3. The ability of an organism to survive an unfavorable environment is a function of its age or maturity.
4. Organisms can survive higher intensities of an unfavorable environment during the resting stage than during the actively multiplying stage.

STUDIES ON NUCLEIC ACID METABOLISM. SAMUEL GRAFF. (College of Physicians and Surgeons, Columbia University, New York 32, N. Y.)

Two general types of nucleic acid are known: the pentose type and the desoxypentose type. Only one of each of these has been characterized, the ribose nucleic acid of yeast and the desoxyribose nucleic acid of thymus. Virtually all other tissues and organisms give rise to one or both general types of nucleic acid, pentose or desoxypentose, but otherwise of unproven constitution. Biological specificity of the nucleic acids is indicated by the behavior of Avery and McCarty's pneumococcus transforming factors, on speculative consideration of Spemann's organizer, and on other grounds. Critical re-examination of the known properties of the nucleic acids suggests the existence of nucleic acid variants, and the constitutional basis of such variation.

The problem of the precursors, biological synthesis and metabolic fate of the nucleic acids is under extensive experimental attack in many laboratories by means of the application of tracer isotopes to a wide variety of biological systems including microbiological mutants, developing amphibian embryos, and mammalian carcinogenesis.

The intracellular distribution of the two types of nucleic acid has been demonstrated by differential stains and by physicochemical and enzymatic methods. It appears that the nucleic acids are not present in free dispersion, but are found only in particles, microsomes, mitochondria, nucleoli, or chromosomes. Evidence that the nucleic acids are not only associated with genes but may comprise an integral part of the genes themselves is accumulating in an impressive manner. On the other hand, little or no evidence has been adduced to suggest what function or role the nucleic acids might play in these particles. The writer offers the hypothesis that the nucleic acids have the function of controlling a large and important group of enzymes. This speculation rises from several known facts including those of the solubility behavior of nucleic acid protein complexes, the salt-like or loose co-valent linkages of nucleic acids with proteins, and the behavior of the nucleic acids in both cellular and protein reproduction.

THE USE OF RADIOACTIVE MANGANESE AND GOLD COLLOIDS IN TREATMENT OF MALIGNANCIES. P. F. HAHN and C. W. SHEPPARD. (Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee)

Radioactive colloids of $Mn^{52}O_2$ and Au^{198} produced in the cyclotron and uranium chain reacting pile respectively have been administered by vein in the treatment of over 100 patients with malignancies, chiefly of the lymphoid-macrophage system. The best results were obtained in the cases of chronic lymphatic leukemia. Remissions in Hodgkin's disease were quite variable in degree and duration. Acute leukemias although responding well in some cases for short periods usually succumbed to hemorrhagic sequelae.

It is too early to assess adequately the value of this particular form of therapy since the work has been in progress only two years. However it appears that the radioactive gold accomplishes the same end as X-radiation in a more convenient and economical manner. There also has been practically no evidence of radiation sickness in the subjects treated.

Radioactive colloidal Au can be infiltrated directly into discrete tumor masses. The metallic ultramicroscopic particles, insoluble in body fluids, remain in situ and offer an excellent means of delivering ionizing radiation from beta particles to the affected tissue with little if any effect on the normal adjacent structures. Several cases of lymphosarcoma, squamous cell carcinoma, and lymphadenopathy due to both leukemia and Hodgkin's disease were treated in this manner with good results. The use of the direct infiltration technique in conjunction with surgery in the treatment of deep lying neoplasms was discussed briefly.

In certain instances in which the tumor tissue was too friable to retain the colloidal solution preliminary attempts to utilize the caustic properties of $Ag^{111}No_3$ were employed.

It is felt that the isotopes of gold and silver because of their peculiar properties will be very useful in tumor therapy.

THE COLLECTION OF RADIOACTIVE IODINE BY BENIGN AND MALIGNANT TUMORS OF THE THYROID. RULON W. RAWSON, BENGT N. SKANSE, REX G. FLUHARTY, and L. MARINELLI. (Massachusetts General Hospital, Boston 14, Massachusetts, and Sloan-Kettering Institute for Cancer Research, New York 21, N. Y.)

The capacity of benign and malignant tumors of the thyroid to collect radioactive iodine has been studied. Tracer doses of radioactive iodine (I^{131}) have been administered to patients having thyroid tumors 24 to 48

hours previous to thyroidectomy. Specimens have been taken from tumorous and normal tissue, weighed and digested in 5 per cent sodium hydroxide solutions. The percentage of administered radioactive iodine collected per milligram of tissue was determined by measuring the radioactivity in aliquots of each digested sample and comparing with measured standards taken from each individual tracer.

It has been observed that in general the capacity of benign tumors of the thyroid to collect radioactive iodine parallels the degree of differentiation observed on histologic examination.

In general malignant tumors of the thyroid have been found to have from none to only a small fraction of the avidity for iodine observed in the normal thyroid tissue.

In an attempt to increase the avidity for radioactive iodine of distant metastases from primary cancers of the thyroid, total thyroidectomy (surgical or with radioactive iodine) has been done in 10 cases. In 5 cases following total thyroidectomy the avidity of metastatic thyroid tissue for radioactive iodine has been significantly increased. In one instance the avidity for radioactive iodine by metastatic thyroid tissue increased considerably after administering thiouracil until the patient developed myxedema.

RADIOPHOSPHORUS THERAPY. B. E. HALL. (Mayo Clinic, Rochester, Minnesota)

This study is based on an analysis of the results obtained with radiophosphorus in the treatment of 124 cases of polycythemia vera, 33 cases of leukemia, 25 cases of multiple myeloma, and 2 cases of Hodgkin's disease, the latter with extensive involvement of the bone marrow. The mode of administration, dosage, results of treatment, complications, advantages, and disadvantages of this form of therapy in each group of diseases listed above were discussed.

In polycythemia vera, satisfactory remissions accompanied by symptomatic improvement were observed in 85 per cent of the patients treated with the isotope, and partial remissions were noted in 8 per cent. The remissions lasted from 6 months to 2 years in most cases after the first course of treatment. Remissions lasting 3 or more years were noted in 2 cases, 4 years in 1 case, and 5 years in 1 case.

Six deaths have occurred among the 124 cases of polycythemia vera; 4 of these resulted from the development of a terminal fulminating acute leukemia, 1 from cerebrovascular accident (no autopsy), and 1 from congestive heart failure.

A diminished incidence of thrombophlebitis and thrombosis in patients during periods of remission also was noted.

In 20 cases of chronic myelogenous leukemia and in 6 cases of chronic lymphatic leukemia, remissions were induced with radiophosphorus in a manner similar to the induction of remissions by means of roentgen irradiation. All of the patients with chronic lymphatic leukemia are living at the present time; 10 of those with chronic myelogenous leukemia are dead, 6 of whom died with a terminal acute leukemia, an incidence of an acute terminal phase occurring among patients with chronic myelogenous leukemia treated by means of x-rays.

Radiophosphorus, employed as the sole therapeutic agent, was ineffective in 4 cases of acute leukemia, 2 cases of Hodgkin's disease, and 12 cases of myeloma. In 12 additional cases of myeloma, intensive roentgen irradiation was administered after treatment with radiophosphorus. In general, these results were poor also, although in 1 instance local regression of multiple tumors followed by bone repair occurred.

THE EFFECTS OF RADIATION ON MAMMALIAN TISSUE CELLS, NORMAL AND MALIGNANT. ANNA GOLDFEDER. (New York University, New York, N. Y.)

By the use of the tissue culture technic the difference in radiosensitivity of cellular components was studied. Thus, the most radiosensitive part proved to be the nuclear substance. Consequently, the cells appeared to be most radiosensitive during mitotic division, at which time the chromatin substance is in the most concentrated form.

The formation of cell anomalies and multinucleated cells, as well as cells having nuclei of various sizes and shapes following radiation was shown.

The selective action of radiation on tissue cells was demonstrated. Thus when all other cell types were destroyed by a given dosage, the renal epithelium as well as that of the tongue persisted in growth.

An equivalent factor between x-rays and gamma rays (r/mgm.-hr.) of 5.8 was obtained from data gathered from experiments with mammalian tissues grown *in vitro*. This factor proved later to correspond to that in which erythema was produced by x-rays and radium on the human skin.

The direct and indirect action of radiation was explained by results obtained from experiments carried out *in vitro* and *in vivo*. For example, a dose of about 60,000 r was required to prevent the growth of mouse Sarcoma 180 grown *in vitro,* while a dose of about 5,000 r was sufficient to produce a similar effect *in vivo*. However, the latter proved to be only the attenuating dose, because the irradiated tumor fragments were able to produce resistance in mice to further viable tumor grafts, when implanted, while tumor implants irradiated with 60,000 r failed to produce this phenomenon.

Similar observations were later made with a reticulum cell lymphosarcoma in rats.

A wide difference in radiosensitivity of two analogous mammary tumors of inbred strains of mice grown *in vitro* and *in vivo* was demonstrated. From the experimental data it was concluded that a histological classi-

fication of tumors is not a sufficient guide when their radiosensitivities are concerned.

A classification regarding biological characteristics of tumors in relation to radiosensitivity, was suggested.

INTRODUCTION TO CHEMOTHERAPY SYMPOSIUM. AIMS AND METHODS. AUSTIN M. BRUES. (Argonne National Laboratory, University of Chicago, Chicago, Illinois)

In looking for chemotherapeutic agents, various approaches are possible. The purely intuitive approach has long been practiced. The logical method, in which analogs and derivatives of agents with known biological or carcinolytic activity are studied, helps to explain mechanisms of action and to gain more powerful agents. The "catalog" approach, in which many substances are tested because they are available, should certainly not be neglected; urethane, for example, might have been turned up decades earlier by this approach, and one wonders to what extent such common chemicals have yet been investigated.

Obviously the ideal test object would be the sum total of the various types of human tumors. For many reasons a simplification of the test object must be used in the search for promising agents, especially in large scale studies, resulting in the use of procedures currently called "screening." Such procedures are inevitably bound to lose in depth as they gain in breadth. Much careful thought has been given to cutting the loss to a minimum, and the present symposium deals with this matter, as well as with some of the current results.

EFFECTS OF METABOLIC PRODUCTS OF FUNGI ON THE VIABILITY AND GROWTH OF CARCINOMA, SARCOMA AND MELANOMA IN MICE. C. CHESTER STOCK, K. SUGIURA, and C. P. RHOADS. (Sloan-Kettering Institute for Cancer Research, New York 21, N. Y.)

A year ago there was started a systematic study for the purpose of securing information on the effects of metabolic products of bacteria and fungi on cancer tissue. Among the abnormal tissues selected for study were mouse sarcoma 180, the Harding-Passey melanoma and the mouse mammary adenocarcinoma E0771. Conditions of tests *in vitro* and *in vivo* were developed for the cultural products. Isolated antibiotics have been tested in addition to tests made upon more than 500 sterile filtrates from cultures of *Penicillia, Aspergilli, Actinomycetes, Basidiomycetes,* some unidentified fungi and a few bacteria.

In experiments *in vitro* the tumor fragments were immersed in culture filtrates and kept at 4 to 5° C. for 24 hours before transplantation. The filtrates (or solutions of pure materials) were adjusted to a pH between 6 and 8 (usually 7 to 7.4) and to a tonicity satisfactory for the tumor. Culture filtrates of *Streptomyces griseus, Actinomyces antibioticus,* of several *Penicillia* and of some unidentified fungi gave 90 to 100 per cent inhibition of the transplantability of Sarcoma 180 and the Harding-Passey melanoma. Culture filtrates of *Aspergillus fumigatus,* various *Basidiomycetes,* of *Trypanosoma cruzi* and of Coley's toxin had no inhibitory effect. Neither crystalline penicillin G (10,000μ/ml.) nor streptomycin (10 mgm./ml.) had any inhibitory action upon the growth of sarcoma 180, the melanoma, nor the adenocarcinoma. Solutions or suspensions of the following gave 80 to 100 per cent destruction of the proliferating capacity of these tumors: gliotoxin (1 mgm./ml.), citrinin (2.5 mgm./ml.), clavacin (1 mgm./ml.) and podophyllin (0.1 mgm./ml.). Rutin (1 mgm./ml.), quercetin (1 mgm./ml.), podophyllotoxin (0.1 mgm./ml.) and cytochrome C (17 mgm./ml.) had little or no inhibitory effect.

For experiments *in vivo* fragments of sarcoma 180 were implanted into mice by the usual trocar method. From 0.1 to 1.0 cc. of the culture filtrates were injected intraperitoneally twice daily for 7 days starting 1 day after tumor implantation. In the case of Harding-Passey melanoma and adenocarcinoma E0771, the treatment continued much longer. Culture filtrates of *Aspergillus fumigatus, Penicillium notatum,* and certain other species of *Penicillia, Actinomyces* and *Streptococcus hemolyticus* plus *Serratia marcescens* showed marked or definite inhibitory action upon the growth of transplanted tumors. Repeated intraperitoneal injections of culture filtrates of *Aspergillus fumigatus, Penicillium* and podophyllin had slight inhibitory action upon spontaneous mammary cancers in mice. Unlike bacterial toxins and bacterial polysaccharides, most of the culture filtrates of *Aspergilli, Penicillia* and other molds tested did not produce tumor hemorrhage.

THE PLACE OF TISSUE CULTURE IN A CANCER CHEMOTHERAPY SCREENING PROGRAM. RICHARD A. ORMSBEE, and IVOR CORNMAN. (The Sloan-Kettering Institute for Cancer Research, New York 21, N. Y.).

Two questions may be asked in considering the use of tissue culture for the screening of compounds. First, can a definite selective damage to cancer cells be detected in tissue culture? Second, can a system for evaluating responses of normal and malignant tissues be applied to tests of a large number of compounds?

The first question can be answered from earlier results with crude penicillin. One can kill the cells of several rat sarcoma cells in a culture tube wherein normal fibroblasts survive (1).

In attempting to answer the second question we have established simple, objective criteria for evaluating cell and tissue changes. Our criteria include (a) the extent of growth, (b) the degree of lysis of the plasma clot, the amount of (c) granularity and (d) rounding and (e) the amount of cell disintegration. Granularity and rounding were scored twice, once for the degree of granularity or rounding shown by the affected cells,

and again for the relative number of cells affected. Each criterion of cell damage was given a grade of 1 to 4 depending upon the degree of effect. The tissues were scored before and after a 24-hour exposure to the agent under test. The work with podophyllin may be cited as an example. In these tests we limited the score to cellular damage (rounding, granularity and disintegration). We found the normal tissue (mouse fetal skin) undamaged by exposure to 12 mgm./L of podophyllin while the "damage scores" for lung tumors MA387 + sarcoma L946 were more than doubled as a result of the 24 hour exposure, giving selective indices of 36 and 41 respectively. Analysis of the damage scores of a large number of such therapeutic candidates indicates that a selective index (damage to malignant tissue minus damage to normal tissue) of 0 to 10 denotes insignificant selective activity; 11 to 20 slight selective action, and 21 and above considerable selective action upon malignant cells.

The assignment of different values to different cytological effects is being explored. Disintegration, for example, obviously indicates greater damage than does mere rounding of the cell. Another manipulation mathematically reasonable is multiplication of the extent of rounding by the relative proportion (in grades of 0 to 4) of rounded cells. By scoring rounding and granularity in this way, *i.e.* degree $\times$ number affected, the preceding tests with podophyllin give a selective index of 64 for the lung tumor and 75 for the sarcoma.

By means of these objective criteria it has been possible to classify a large number of compounds as selectively or non-selectively damaging to malignant cells in tissue culture in comparison with normal fetal mouse skin cells. The results to date show that approximately 2 per cent of the substances so far tested have selective indices that we consider significant.

REFERENCE

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SCREENING OF CHEMICAL AGENTS FOR CAPACITY TO DAMAGE TUMORS *IN VIVO*.

VIRGINIA DOWNING. (Chemotherapy Section, National Cancer Institute, Bethesda 14, Maryland).

The ultimate objective of our group is to develop some chemical agents which, either by themselves or in combination with surgery and radiation, will control tumors without causing severe and irreparable damage to normal tissues. Since there are countless chemicals available or possible of synthesis, these screening procedures were designed to pick out a small number with demonstrated potency in damaging tumors with which to carry out further experiments, including attempts at regression.

The several steps in this selection procedure are:

Vehicle.—The most appropriate solvent or vehicle for each chemical agent is first determined; *viz.* water (with weak acid or alkali if necessary), oils, propylene glycol, and the like.

Maximum tolerated dose.—The largest single subcutaneous dose tolerated by normal mice is ascertained.

First screening.—Fifteen mice bearing week-old intramuscular implants of sarcoma 37 are given a single maximum tolerated dose subcutaneously at a site distant from the tumor. After 8, 24, and 48 hours, these mice are killed, 5 at each period, together with untreated controls. Tumors are fixed in Zenker-Formol fluid and also other tissues (intestine, kidney, liver, etc.) if indicated. The extent and type of induced damage are ascertained by microscopic examination.

Second screening.—Maximum tolerated dose is determined with greater precision using larger numbers of tumor-bearing mice. The time of maximum effect and the minimum effective dose, following a single injection of each active agent, are also determined in tumor-bearing mice.

Third screening.—Effects of these active agents on other types of tumors, morphologic effects of repeated doses on tumors and other tissues, and chronic toxicity are being investigated.

Attempts at regression.—These will be done with the few agents that survive these screenings.

THE NITROGEN MUSTARD DERIVATIVES IN THE CHEMOTHERAPY OF TRANSMITTED LEUKEMIA IN MICE.

J. H. BURCHENAL, J. B. RILEY, and R. A. LESTER. (Sloan-Kettering Institute for Cancer Research, New York 21, N. Y.)

The method of Flory, Furth, Saxton, and Reiner, using transmitted mouse leukemia as a screening procedure in the evaluation of unknown compounds has been studied and revised with a view to use in a large scale screening program. All compounds are tested against the same two strains of myeloid (Ak 1394) and lymphoid (Ak 9417) leukemia. Prolongation of survival time is used as the criterion of the chemotherapeutic activity of a drug.

In the usual experiment, 120 Akm mice are injected intraperitoneally with 0.1 cc. of a suspension of a leukemic spleen. Forty-eight hours later these animals are divided into 12 groups of 10 mice each (one set of untreated controls, one set of controls treated with a standard active compound, and ten sets of mice treated with unknown compounds). All compounds are given intraperitoneally in maximum tolerated doses thrice weekly for 10 doses. Groups of mice treated with either of the standard active compounds, HN 2-(methyl-*bis*-[2-chloroethyl]-amine) or SK 137 (1,3 propane diamine, 2-chloro-N, N, N′, N′-tetrakis [2-chloroethyl]), usually show an average increase in survival time of about 100 per cent over the untreated controls. The chemotherapeutic effect of unknown derivatives in

relation to that of the standard compounds can be compared by this method.

In screening the nitrogen mustard derivatives certain relationships of activity to chemical structure were noted. In the small number, 30 in all, tested to date, the quaternary ammonium compounds and those containing only a single 2-chloroethyl group were inactive. Nitrogen mustards with two 2-chloroethyl or 2-bromoethyl groups were usually chemotherapeutically active. Increasing the substituent group from the methyl through ethyl, n-propyl, n-butyl, etc. to n-hexadecyl, decreased toxicity and allowed a thousand-fold increase in dosage. There was, however, no concomitant increase in therapeutic effect. The diamine mustards with four 2-chloroethyl groups were particularly effective in some experiments.

Five compounds of chemotherapeutic activity equal to or greater than that of methyl-*bis*-(2-chloroethyl) amine (HN 2) were uncovered by this procedure.

SK 107 1,2 ethane diamine N, N, N′, N′-tetrakis (2-chloroethyl)
SK 136 1,3 propane diamine N, N, N′, N′-tetrakis (2-chloroethyl)
SK 137 1,3 propane diamine, 2-chloro N, N, N′, N′-tetrakis (2-chloroethyl)
SK 113 *bis* [*bis* (2-chloroethyl) amino ethyl] sulfide
SK 437 methyl *bis* (2-bromoethyl) amine

Of the miscellaneous compounds tested by this method, colchicine and ethylcarbamate were active but all the more complicated carbamates were inactive.

THE USE OF SEX HORMONES IN ADVANCED MAMMARY CANCER.

R. C. MELLORS,* F. E. ADAIR, G. C. ESCHER,* J. H. FARROW, and H. Q. WOODARD. (Sloan-Kettering Institute for Cancer Research, and Memorial Hospital for Treatment of Cancer and Allied Diseases, New York 21, N. Y.)

This paper constitutes a preliminary presentation of work now in progress dealing with the use of sex hormones in advanced mammary carcinoma. To date, 89 female patients have received either male or female sex hormone, dependent upon certain indications, principally the patient's age (estrogen being used only after the menopause), and the site of the metastases (androgen being used principally in the presence of osseous metastases). The dosage has been: androgen, testosterone propionate, 100 mgm., I.M., 3 times a week; diethylstilbestrol, 15 mgm. daily; ethinyl estradiol, 3 mgm. daily; estrone sulfate, 30 mgm. daily.

Of 53 patients receiving androgen, only 36 are considered to have been studied for sufficient time. Of these, 8 have shown temporary regression or inhibition of tumor metastases (4 showed increase in calcification and decrease in size of osseous metastases; 1, decrease in size of pulmonary metastases; 1, disappearance of pleural effusion; 1, cessation of menorrhagia due to uterine metastases; 1, disappearance of jaundice due to common duct obstruction by metastases to lymph nodes). At this writing, 3 of the cases have progressed.

Of thirty-six patients receiving estrogen, twenty are considered to have been followed for an appropriate time. Of these, 5 have shown a decrease in size of cutaneous metastases or local tumor recurrences. At this writing, one of these has progressed.

In these therapeutic trials, emphasis must be placed upon the untoward as well as the beneficial reactions. The untoward reactions may include the possibility of accelerating mammary cancer growth by the use of estrogen in younger women, and of promoting osteolysis and concomitant hypercalcemia by the use of androgens.

A number of laboratory studies, designed to give information concerning the mechanism of action of sex hormones in metastatic mammary cancer, have been carried out. Observations, admittedly limited, which pertain to the cancer cell and its immediate environment include the following: cytological studies of regressing metastatic mammary cancer cells rarely show evidence of a "cytotoxic" effect (condensation and distortion of nucleus, etc.); histochemical studies (alkaline phosphatase activity, high polymer sulfuric acid ester distribution), again limited, indicate a considerable biochemical alteration of the stroma, related, it would appear, to proliferative activity of fibroblasts. Further study is required to determine whether this stromal reaction is related to the declining growth rate of cancer cells, the desmoplastic activity of the cancer cells, or to the effect of estrogen.

THE ROLE OF PHARMACOLOGY IN THE CHEMOTHERAPY OF CANCER.

FREDERICK S. PHILIPS. (Sloan-Kettering Institute for Cancer Research, New York 21, N. Y.)

Pharmacology can contribute generously to programs designed to discover and develop chemotherapeutic procedures useful in the treatment of neoplastic diseases. Prior to screening for activity against experimental tumors, studies of the toxicity of candidate compounds should be undertaken by investigators experienced in toxicological analysis. Thereby reliable information can be provided concerning the maximum dosages likely to be tolerated by laboratory animals harboring experimental tumors. Such technics can also elucidate optimal methods for the administration of candidate agents.

When the activity of new compounds against experimental neoplasms is sufficient to warrant their clinical trial, it is essential that they be subjected to thorough pharmacological analysis. Laboratory studies should endeavor to provide the clinician with a rational basis for the selection of initial dosages to be ad-

* This work was done in part under a Fellowship grant of the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

ministered to human beings. Close scrutiny of the actions of new compounds in experimental animals should prepare the clinician to deal with undue manifestations of toxic action in human patients. Furthermore, pharmacological studies may also have provided means whereby the manifestations of intoxication could be antidoted or antagonized. Finally, unexpected drug actions which become apparent during preliminary human trials may necessitate additional pharmacological investigations with experimental animals. Clearly, the cooperation of the pharmacologist and the clinician in the application of new drugs to the chemotherapy of human neoplasms will do much to reduce the hazards attending their administration to human beings.

The potential contribution of pharmacology to the control of neoplastic diseases is not limited to the development of new methods of chemotherapy. Intensive study of the mechanism of action of drugs which have been shown to effect a significant change in the course of neoplastic diseases may in itself provide greater understanding of the nature of the diseases. In support of this contention it need only be mentioned that the discovery of new and potent drugs has in the past often led to greater understanding of the normal physiology of cells, tissues, or organs specifically responsive to the action of the new agents.

THE CLINICAL EVALUATION OF NITROGEN MUSTARD DERIVATIVES IN LEUKEMIA.

J. H. BURCHENAL,* D. A. KARNOFSKY, A. J. ERSLEV, L. F. CRAVER, and C. P. RHOADS. (Sloan-Kettering Institute for Cancer Research, New York 21, N. Y.)

Leukemia presents an unique opportunity for the evaluation of chemotherapy in as much as the neoplastic cells can be observed repeatedly at any desired time before or after treatment. According to the criteria set up at Memorial Hospital for the clinical evaluation of chemotherapeutic agents, an ideal response would be as follows: the white blood count would fall, the hemoglobin and platelets rise to normal values and the differential return to a normal distribution. The spleen and lymph nodes would decrease to normal size, the fever and hemorrhagic tendency disappear, and the basal metabolic rate fall. With the ideal drug such remissions would be permanent or at least repeatable.

A nitrogen mustard derivative, SK 136, 1:3 propane diamine, N, N, N′, N′-tetrakis (2-chloroethyl) has been tested by such criteria. The dosage of 0.1 mgm./kgm. daily for 4 to 8 doses was comparable to that of HN 2, methyl-*bis*-(2-chloroethyl) amine. In 15 cases of acute leukemia, 15 showed a fall in white cell count, and 3 a rise in hemoglobin and platelets, a differential which returned to normal and a decrease in bleeding. In 8 the spleen and in 10 the nodes decreased in size. Eight of 15 showed some evidence of subjective improvement coincident with therapy with SK 136 but in only 3 was the remission complete and in none was it repeatable. The duration of these remissions was from 1 to 3 months.

No apparent benefit was noted in the 2 cases of chronic lymphatic leukemia treated with this drug.

Eight patients of chronic myelogenous leukemia have been treated with SK 136. In all 8 there was a decrease in white cell count, to within normal limits, 6 out of 7 a rise in hemoglobin (1 more had normal values before and after therapy), 6 a decrease in size of the spleen and 7 a definite subjective improvement. In these patients there was almost no nausea and vomiting and only a mild degree of dizziness. Although the average remission of 2½ months was somewhat shorter than would ordinarily be expected from x-ray therapy, the last x-ray-induced remission in four of the cases had been no longer than this. Two patients who had been resistant to x-ray therapy and 3 who had not been benefited by Fowler's solution responded to this treatment.

Therapy with SK 136 in acute leukemia, although occasionally promising, has generally been unsatisfactory, but in chronic myelogenous leukemia, although not curative, it appears to be a useful tool in the armamentarium of the clinician.

* This work was done under a Fellowship grant of the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

OBSERVATIONS ON THE CLINICAL RESPONSE AND HISTOPATHOLOGIC REACTION OF LYMPHOMA TO NITROGEN MUSTARD THERAPY.*

CHARLES L. SPURR, MATTHEW H. BLOCK, T. R. SMITH, and L. O. JACOBSON. (Department of Medicine, University of Chicago, Chicago 37, Illinois)

Observations on the clinical application of the nitrogen mustard compounds to therapy of various lymphomas and malignant blood dyscrasias have been extended toward four objectives: (a) to clarify the histopathologic changes in the bone marrow; (b) to ascertain the regenerative capacity of the marrow following repeated courses; (c) to determine the cytological events in the tissue following treatment; (d) to observe the long range response to repeated treatment with HN_2, alone and as an adjunct to roentgen therapy.

The events following HN_2 therapy have been studied in rib or sternal marrow comparing pre-treatment and post-treatment biopsies. These usually show the cytotoxic effects within 96 hours following the first of 4 intravenous injections of 0.1 mgm./kgm. body weight. The principal injury is to the promyelocyte and myelocyte stages; in the erythroid series less degeneration occurs. This initial phase is followed by an atrophy of the marrow in the second and third week in which the granulocytic precursors and to a less degree the ery-

* This work was supported in part by a grant from the Committee on Growth of the National Research Council acting for the American Cancer Society.

throid series are depressed. Recovery is orderly and in normal sequence with myeloid and erythroid regeneration increasing in the third to sixth week. These events are identical to those described from sternal aspiration smears.

A survey of the reaction of the peripheral blood to repeated doses of HN_2 has been extended in order that we may detect any evidence of a cumulative effect leading to a hypoplasia of the marrow. To date we have reached cumulated doses of 350 mgm. and find that the pretreatment, post-treatment leukocyte and erythrocyte counts are within normal limits although the percent of leukopenias of less than 3,000 is increased. This may be due to the advanced stage of the disease rather than a toxic effect of the nitrogen mustard.

The histopathologic changes in the various lymphomas following intravenous injections of HN_2 have been observed in a series of pretreatment and post-treatment biopsies. The alterations following treatment are difficult to assess because of the constant spontaneous degeneration occurring in these tumors. The most conspicuous changes are seen in tumors that retain a follicular structure with germinal centers. In the follicular lymphoblastoma, and to a less degree in the giant follicular lymphoma, there is visible degeneration of the medium lymphocytes with fragmentation of the chromatin and later the nuclei; the cytoplasm becoming darker. This is apparent within 24 hours following a dose of 0.1 mgm./kgm. body weight. In the fourth to 12th days following 4 injections of 0.1 mgm./kgm. body weight there is a decrease in their number of small lymphocytes. The duration of this atrophic period is allied to the growth rate of the tumor and may vary from a few days to several weeks.

Similar cellular changes were observed in the Hodgkin's tissues. The degree of change was related to the histopathology of the node with less acute cellular degeneration and a shorter atrophic period in the sarcomatous group.

In cases of multiple myeloma, advanced myelogenous leukemia and an anaplastic carcinoma no significant histological changes were seen. There was an increase in plasma cells and eosinophiles following treatment.

Clinical observations on the lymphomas are still too brief to discuss results in terms of longevity. In the initial group of 27 cases of Hodgkin's disease, 9 are well, 3 are invalids, 14 have died. Two principal problems presented as the cases terminated; (a) a myelophthisic anemia made the use of cytotoxic HN_2 undesirable, (b) the rapid regeneration of tumor following treatment resulted in remissions which were clinically insignificant. Remissions in "roentgen resistant cases" apparently are due to the systemic nature of HN_2 therapy as compared to local roentgen therapy. Fourteen cases in this catagory were treated: 7 responded and are well 10 to 24 months after initial HN_2 injection; 5 cases had remissions of 3 to 10 months, death following; 2 failed to respond. The clinical response in the other lymphomas and in Hodgkin's sarcoma appears to depend upon the growth rate of the tumor. There is no evidence that HN_2 will alter the basic histological characteristic of the tumor.

THE EFFECTS OF CHLORETHYLAMINES ON TUMORS. E. BOYLAND. (Chester Beatty Research Institute, Royal Cancer Hospital [Free], London, England)

Chlorethylamines such as methyl bis (β-chlorethyl) amine (HN_2) have an effect similar to that of urethane and of radiations on mitosis in dividing cells. Urethane and chlorethylamines can therefore be described as radiomimetic drugs. Cytological examination of animal and human biopsy material following treatment with HN_2 has shown abnormal mitosis similar to those seen following x-irradiation of tumors. Favorable effects have been obtained in the treatment of rats bearing the Walker carcinoma and of C3H mice with a transplanted lymphosarcoma by giving weekly doses of 1 mgm. per kgm. of HN_2. Cases of bronchogenic carcinoma treated with chlorethylamines have shown evidence of clinical improvement including relief of pain and dyspnoea in the majority of cases, although actual reduction in tumor size is not common.

THE EVALUATION OF CHEMOTHERAPEUTIC AGENTS AGAINST NEOPLASTIC DISEASE. D. A. KARNOFSKY, and J. H. BURCHENAL. (Sloan-Kettering Institute for Cancer Research, New York 21, N. Y.)

A number of substances have been proposed for clinical use in the treatment of neoplastic disease. This list includes *radioactive isotopes*—phosphorus, sodium, iodine, manganese and gold; *hormones*—estrogens and androgens; *chemicals*—nitrogen mustards, urethane, benzene, potassium arsenite, chemicals interfering with carbohydrate metabolism, stilbamidine, colchicine, folic acid derivatives; and *biological preparations*—KR, bacterial polysaccharides, Coley's toxin, ACS and tyrosinase. As interest in the field of cancer chemotherapy increases, the list will become longer.

The clinical trial of these and other agents is justified for investigative purposes. It has become apparent, however, that the results of these research studies are sometimes anticipated or exaggerated, with undue and unjustified provocation of the laity and medical profession. A procedure for clinically evaluating the effects of chemotherapeutic agents against cancer was, therefore, briefly proposed at the conference.

Any medical institution is permitted to test clinically new drugs, including chemotherapeutic agents against cancer. In the latter instance, previous laboratory data on the effects of the drug on animal tumors and toxicological data should be available before the test is undertaken. In carrying out the first clinical tests, the following points should be considered.

1. A knowledge of the natural history of the disease in man.

2. Careful selection of the patients; so that results are not confused by other types of therapy or unrelated complications.

3. A careful analysis of the subjective criteria of effect of the drug.

4. A careful analysis of objective criteria of effect of the drug. Laboratory tests whose results, interesting though they may be, cannot be correlated with clinical improvement, should not be included in the clinical assessment.

5. An objective analysis of the patient's ability to care for himself and to do useful work. The suggested system of evaluation is called the *General Performance Status,* and the criteria used in our clinic are as follows:

		GPS Percentage	
A	Able to carry on normal activity and to work. No special care is needed.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity, minor symptoms or signs of disease.
		80	Normal activity with effort, some signs or symptoms of disease.
B	Unable to work. Able to live at home, care for most personal needs. Varying degree of assistance is needed.	70	Cares for self, unable to carry on normal activity or to do active work.
		60	Requires occasional assistance, but is able to care for most of his needs.
		50	Requires considerable assistance and frequent medical care.
C	Unable to care for self. Requires equivalent of institutional or hospital care. Disease may be progressing rapidly.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization is indicated although death not imminent.
		20	Hospitalization necessary, very sick, active supportive treatment necessary.
		10	Moribund, fatal processes progressing rapidly.

6. A measure of the duration and nature of any remission.

7. The prolongation of life by the use of the agent.

8. The value of the agent in preventing or treating expected complications of the disease?

If the testing group can develop clear, definite and unequivocal evidence that the agent has produced some useful effect in the treatment of cancer, a preliminary report should be prepared. Unless the agent has a therapeutic advantage in certain situations beyond that possessed by accepted forms of treatment, the material should be presented very conservatively.

If the clinical data indicate that the new agent may be of value in the treatment of cancer, it should then be studied cooperatively under controlled conditions by a number of institutions on a large number of patients in all stages of the disease. The results of this study should be reviewed by a central group. The final report should contain clear and authoritative statements as to the role of the new drug in the treatment of cancer.

THE EFFECT OF VARIOUS AGENTS ON THE GROWTH OF SARCOMA 180 ON THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO, D. A. KARNOFSKY, L. M. PARISETTE and P. A. PATTERSON. (The Sloan-Kettering Institute for Cancer Research).

The growth of the sarcoma 180 on the chorio-allantoic membrane of the chick embryo, and the technic for testing the ability of various chemicals to modify this growth was described at the A.A.A.S. conference in 1946. In the past year the maximum tolerated dose for the 12 day-old chick embryo was determined for a number of substances, and these agents were tested for chemotherapeutic activity against sarcoma 180. The results are tabulated below.

Agent	Max. tolerated dose/egg		Chemotherapeutic Effect*
Nitrogen Mustards			
HN_2 (SK101)	0.3	mgm.	+
HN_3 (SK100)	0.3	"	+
SK 107	1.0	"	+
SK 136	0.3	"	+
SK 137	1.0	"	+
SK 500	0.5	"	+
SK 501	0.5	"	+
SK 502	3.0	"	+
SK 437	1.0	"	+
Mustard sulfone	0.03	cc.	+
Urethane	100.0	mgm.	0
Colchicine	0.015	"	0
Potassium arsenite	0.15	"	0
Heptaldehyde	0.025	cc.	0
Stilbamidine	0.8	mgm.	0
Teropterin	20.0	"	0
Podophyllin (crude)	0.005	"	0
Podophyllin (alkali treated)	3.50	"	0
Podophyllotoxin	0.0015	"	0
Quercetin	10.0	"	0
Gliotoxin	0.050	"	0
Coley's toxin	0.20	cc.	0
X-rays	500	r	+

* + indicates that the agent shows some evidence of chemotherapeutic activity. 0 indicates that the agent shows no evidence of chemotherapeutic activity.

The nitrogen mustard homologues tested have consistently shown chemotherapeutic activity. Of the other agents tested, x-rays and di-(β-chloroethyl) sulfone are the only ones showing some effect on sarcoma 180 at doses that are not lethal to the chick embryo.

CARCINOGENIC ACTION OF 2-ACETAMINOFLUORENE. K. E. PASCHKIS, and A. CANTAROW. (Jefferson Medical College, Philadelphia 7, Pennsylvania)

A brief historical review on the discovery of the carcinogenic action of acetaminofluorene (AAF) is given. Work has been done chiefly on rats but malignant growths in a considerable number of tissues have been obtained following oral administration of this carcinogen also in mice, cats and fowl. Data on sex and strain differences are presented. In our own work estrogens (exogenous: estradiol diprop., endogenous: PMS) and androgens (exogenous: testosterone prop., endogenous: chorionic gonadotropins) were given to rats receiving AAF, in the hope of producing carcinoma in the respective target organs. While this did not occur, it was found that the development of cancer of the liver was enhanced by treatment with androgens (in males and females) and estrogens (in females but not in males).

Thiouracil given to rats receiving AAF increased the incidence of tumors of the thyroid (confirming Bielschowsky) and decreased the incidence of liver cancer. Simultaneous administration of thyroxin seemed to invalidate to some degree the protective action of thiouracil. Thiouracil also afforded protection to the liver against the carcinogenic action of AAF enhanced by testosterone prop.

Progesterone given to female rats receiving AAF considerably increased the early incidence of cancer of the breast, whereas estrogens failed to enhance development of mammary cancer.

In animals treated with AAF testosterone was less androgenic and at the same time (co-) carcinogenic: this was reversed by thiouracil. Also preliminary experiments showed that there is a marked difference of 17-ketosteroid excretion. The working hypothesis is therefore suggested that under the influence of AAF the metabolism of testosterone is perverted to a (co-) carcinogen which is weakly androgenic. This perversion of the metabolism can be prevented by thiouracil.

SOME OBSERVATIONS ON CARCINOGENICITY, DISTRIBUTION AND METABOLISM OF *N*-ACETYL-2-AMINOFLUORENE IN THE RAT. H. P. MORRIS, B. B. WESTFALL, CELIA S. DUBNIK, and THELMA B. DUNN. (National Cancer Institute, Bethesda 14, Maryland)

N-Acetyl-2-aminofluorene induces in the rat a wide variety of malignant tumors remote from the site of application. It is carcinogenic either by painting or ingestion.

Mono- and diacetyl-2-aminofluorene were more carcinogenic than 2-nitro, and 2-aminofluorene after topical application as well as after ingestion.

A quantitative estimation of the carcinogen has been carried out by diazotization in strongly acid media and subsequent coupling under alkaline conditions with sodium 2-napthol 3,6-disulfonate to give a red dye. Detectable quantities, using this method of estimation of the compound, were found in the blood plasma 1 hour after administration of 3 to 10 mgm. per 100 gms. body weight in coconut oil by stomach tube. Blood concentration increased up to 3 to 4 hours, following which, at the highest dose level a gradual decrease in concentration occurred over a 16 hour period, while the lowest level of administration none was found in the plasma after 8 hours. Considerable amounts were found in the liver, kidney, none in the red cells, and detectable quantities in muscle, brain and testes. Urinary concentration after a single dose roughly paralleled plasma concentration.

About 25 to 30 per cent of a thrice weekly ingested dose was recovered in the urine after a 7 day period. The compound appeared in the urine in a conjugated form 8 to 10 times more concentrated, when calculated as the acetyl derivative, indicating a change during metabolism that left the amino group still intact. Diazotizable material could not be detected in the urine, beyond 9 days after the last dose.

More tumors and of a greater variety were obtained on synthetic diets containing 18 and 24 per cent casein than on a diet of 12 per cent casein or on a 12 per cent casein diet plus injectable liver extract.

STUDIES ON THE DEVELOPMENT OF SEBACEOUS GLANDS IN MICE AND THEIR ROLE IN EPIDERMAL CARCINOGENESIS. V. SUNTZEFF, C. CARRUTHERS, and E. V. COWDRY. (Research Department, Barnard Free Skin and Cancer Hospital, St. Louis 3; and Department of Anatomy, Washington University Medical School, St. Louis 10, Missouri)

These studies deal in large part with the role of sebaceous glands and hair follicles on the development of skin cancer in very young mice. Previous investigations revealed that baby mice (2 to 10 hours after birth) were refractory to a single application of methylcholanthrene, and an examination of the histogenesis of the sebaceous glands and hair follicles showed that these structures were rudimentary at the time of application of the carcinogen and appeared to be nearly completely developed at 72 hours after birth. The lack of responsiveness of the baby mice may have been due to the absence of sebaceous glands, or to a lack of a portal of entry of the carcinogen via the hair follicles. The studies of Simpson and Cramer indicate that the sebaceous glands play an important role in the genesis of skin cancer in mice (1).

In another experiment the epidermal total lipid, cholesterol, and lipid phosphorus contents of mice at 2, 3, 5, 7 and 14 days after birth and at the age of 1 and 2 months was determined. These lipid constituents remained practically constant until 7 days after birth. At 14 days after birth the cholesterol and lipid phosphorus was essentially normal while the total lipid content had increased but was much less than normal.

In another study, mice from 12, 24 and 72 hours, and from 1, 2, 4, 6 and 8 weeks after birth were treated with one application of methylcholanthrene, and to date (11 to 18 months after the experiment was started) mice younger than 2 weeks have not developed tumors while those older than 2 weeks have. Hence, there appears to be a direct relationship between the susceptibility of the epidermis and the development of the sebaceous glands as indicated by the increased production of cholesterol and phospholipid, both of which may be essential for the carcinogenicity of the hydrocarbon.

In another phase of our program on aging and carcinogenesis the calcium, total lipid, copper and zinc contents of the epidermis of young (2 to 3 months) and old (12 to 13 months) mice, of the New Buffalo and CBA strains were determined. A previous study had revealed that the response of the young and old of the New Buffalo strain was quite different, in that the young developed tumors more quickly and in a higher percentage than did the old, whereas there was no essential difference in both age groups of the CBA strain. However, the difference in response in the strains of mice was not associated with the calcium, total lipid, or copper and zinc contents.

Finally a brief discussion on the role of calcium in growth and in epidermal carcinogenesis in mouse and man was given.

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THE INFLUENCE OF MUTANT CHONDROGENES AND VIRUSES ON PLANT CELLS.

M. W. WOODS, and H. G. Du BUY. (Department of Botany, University of Maryland, College Park, Maryland)

Plant plastids develop from the mitochondrial elements of the cell. The hereditary components of plastids, the chondrogenes, may undergo so-called spontaneous mutation. This mutation rate is ordinarily very low. However, certain recessive nuclear genes can greatly increase it. In plants of *Nepeta cattaria,* homozygous for such recessive mutagenic factors, a wide variety of mutant plastid types appear. These mutant plastid types can be propagated true to character in F_1 hybrids maintained by vegetative reproduction. Such F_1 hybrids, obtained by crossing the originally variegated females with normal green males, are heterozygous for the mutagenic nuclear gene (or genes), therefore further plastid mutation does not occur in them at an appreciable rate. Mutant plastid types are inherited via cytoplasmic (non-mendelian) inheritance. At least six, and probably many more, distinctly different mutant plastid types have been obtained from *Nepeta.* Each of the mutant plastid chondriogene types produces characteristic cellular abnormalities. These may include (a) changes in the structure and function of the mutant plastid, (b) occasional abnormalities in the normal plastids that may be in the same cell, and (c) disturbances in the growth and function of the cell as a whole. Although mutant chondriogenes of *Nepeta* induce pathological cellular changes which closely resemble those produced by certain viruses they are not infectious, that is, they are not transferred from plant to plant by grafting or mechanical inoculation. Mutated plastids may co-exist in the same cells with normal plastids. In succeeding cell generations, however, one or other of the plastid systems may ultimately dominate the cell linea. Certain strains of tobacco mosaic virus induce changes in the structure and function of plastids which closely resemble those associated with mutant chondrogenes. Other cellular abnormalities are likewise similar in both types of disease.

PARASITIZATION OF MOUSE SARCOMA 180 BY VACCINE VIRUS AND ITS EFFECT ON TUMOR GROWTH.

JOSEPH C. TURNER. (Presbyterian Hospital, New York 32, N. Y.)

A strain of vaccinia that was adapted to mice has been shown to be capable of infecting sarcoma 180. Within the tumor the virus may attain high concentrations (titres 10^{-7} to 10^{-9}) which can persist for long periods of time without causing obvious injury to the host. Upon transplantation tumors carrying large amounts of virus grow more slowly and less successfully than they otherwise would. Infected grafts have been found to weigh less than half as much as controls after 11 days of growth, and they have been observed to regress more frequently.

The explanation is offered that the virus acts directly on the neoplastic cells. The mechanism of action is not clear, and may involve the operation of a viral toxin or a competitive antagonism between virus and the infected neoplastic cells.

Increased Weight of the Liver in Wistar Albino Rats with Induced and Transplanted Tumors

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In a report on changes with age in the livers of Wistar albino rats (4), it was pointed out that the average weight of the livers of old (600 to 1,000 days) females with spontaneous tumors elsewhere in the body was significantly heavier than that of tumor-free old females, and of younger females of comparable body length. In old male rats, in which growths rarely occurred, there was no such increase in the average weight of the liver. Comparison of the weight of mammary gland tumors with the weight of the host's liver revealed no correlation, except that enlargement of the liver was found when the weight of the growth exceeded 30 gm.

The work presented here was undertaken to determine whether the apparent influence of tumors on the weight of the liver, as indicated above, was confined to spontaneous growths and occurred only in females, and whether it was correlated with old age.

PROCEDURE

Male and female Wistar albino rats were used. The experimental animals bore subcutaneously either a transferred fibrosarcoma, or a primary tumor induced by injection of methylcholanthrene. The growths did not metastasize. The exact ages of the rats were not calculated, but none was older than 300 days at death. When the animal was killed, the body length from nose to anus was measured, and the subcutaneous tumor, the carcass without the tumor, and the liver, were weighed. The weight of the liver relative to body weight and to body length was calculated. Corresponding control values were taken from the 21 to 300 day old rats dissected for the study of aging (4).

RESULTS

The data for the experimental female animals are presented in Table I, and for the males, in Table II. The rats are listed in order of increasing

TABLE I: WEIGHT OF TUMOR AND OBSERVED AND RELATIVE WEIGHT OF THE LIVER IN FEMALES

Rat	Body length, mm.	Body weight (less tumor), gm.	Tumor weight, gm.	Liver weight, gm.	Liver / B.L.	Liver / B.Wt.
1	202	231	Neg. †	8.65	0.0428	0.0374
2	225	342	2	10.78	0.0479	0.0315
3	214	270	5	9.39	0.0439	0.0348
4	207	201	6	9.30	0.0449	0.0462
5	176	151	10	7.53	0.0428	0.0499
6	175	138	13	7.04	0.0402	0.0510
7	217	210	27	9.28	0.0428	0.0442
8	207	244	32	12.94	0.0625	0.0530
9*	143	81	36	5.26	0.0368	0.0650
10	196	222	40	11.29	0.0576	0.0512
11	188	174	46	9.58	0.0510	0.0550
12*	167	98	60	7.72	0.0462	0.0787
13	213	239	60	12.60	0.0592	0.0527
14	199	197	61	12.06	0.0606	0.0610
15	207	212	62	14.28	0.0690	0.0673
16	201	204	71	11.58	0.0578	0.0567
17	211	230	71	19.12	0.0906	0.0831
18	194	166	81	11.32	0.0584	0.0682
19	200	200	84	10.64	0.0534	0.0534
20	199	211	89	13.37	0.0672	0.0633
21	191	173	95	11.69	0.0612	0.0676
22	200	185	111	11.98	0.0599	0.0686
23	211	265	123	14.38	0.0681	0.0542

* Body length less than 180 mm.
† Tumor inoculated, but failed to grow.

tumor weight. The individual weights of the liver divided by the body length in control and experimental rats are plotted against the body length in the graphs.

From Table I it may be seen that no consistent relationship existed between the weight of the tumor and the actual weight of the liver, or its weight relative to body weight. With the exception of two rats (marked with an asterisk), the liver values relative to body length were greater than 0.0500 when the tumors weighed more than 30 gm. Further growth of the tumor was not accompanied by a proportional increase in liver size. The graph for the females shows that the liver weights of animals with tumors under 30 gm. lay within the range established by the normal controls. The two rats with heavier tumors but normal relative liver weights were small animals, with body lengths less than 180 mm. The relative weights in the remaining female hosts were clearly higher than normal.

Less clear-cut but similar results were found in male rats. The liver weights relative to body length were greater than 0.0500 when the tumors weighed more than 30 gm. in all but 4 of the experimental animals (Table II). Three of these were small animals, with body lengths less than 180 mm. In the graph it may be seen that while some livers were very heavy compared with control values, there is a considerable spread of the relative weights, and overlapping with the control group. However, the average relative weight of the livers of tumor-bearing males (over 190 mm. body length) is 0.0632 ± 0.0021, and of control males of the same range of body length, 0.0499 ± 0.0010; the standard error of the difference between the means being 0.0023, the actual difference is significant statistically.

DISCUSSION

The results of this experiment extend the earlier observations (4) on old female rats with spontaneous tumors to include adult animals of both sexes with induced and transplanted neoplasms. The relationship between the presence of a tumor and an increase of liver weight is clearer, however, in female rats than in males.

There are several interesting parallels with the results obtained by McEwen and Haven (1): (a) These authors, using young male rats inoculated subcutaneously with carcinosarcoma 256, found a significant increase in the percentage of water in the liver, providing the tumor exceeded a critical weight (10 gm.). (b) The percentage of water did not increase with further growth of the tumor. (c) The weight of the liver relative to body weight did not differ significantly from the control figures. In the calculations reported in the present paper, the use of body length rather than body weight as a measure of size revealed a relation between the

TABLE II: WEIGHT OF TUMOR AND OBSERVED AND RELATIVE WEIGHT OF THE LIVER IN MALES

Rat	Body length, mm.	Body weight (less tumor), gm.	Tumor weight, gm.	Liver weight, gm.	Liver / B.L.	Liver / B.Wt.
1	176	133	12	6.64	0.0377	0.0499
2	165	110	26	7.56	0.0458	0.0688
3	162	293	27	5.52	0.0341	0.0594
4	216	288	31	13.84	0.0641	0.0408
5	221	311	32	13.36	0.0605	0.0430
6*	172	131	33	6.47	0.0376	0.0571
7	180	197	39	14.89	0.0827	0.0756
8	217	293	42	14.74	0.0680	0.0503
9	216	255	42	12.16	0.0563	0.0477
10	204	220	42	11.20	0.0549	0.0509
11	213	264	43	11.51	0.0540	0.0436
12	185	217	44	15.75	0.0851	0.0726
13	220	332	44	16.40	0.0745	0.0494
14*	171	129	46	7.19	0.0421	0.0557
15*	159	89	47	4.93	0.0310	0.0548
16	211	246	48	12.05	0.0571	0.0490
17	228	327	50	15.62	0.0685	0.0477
18	221	293	54	14.07	0.0637	0.0480
19	198	206	60	9.09	0.0459	0.0441
20	202	194	67	11.76	0.0582	0.0605
21	194	189	72	11.57	0.0597	0.0612
22	205	224	75	13.85	0.0676	0.0618
23	206	206	81	13.07	0.0634	0.0634
24	215	282	83	17.81	0.0829	0.0632
25	226	319	96	16.59	0.0734	0.0520
26	219	245	126	14.22	0.0649	0.0581

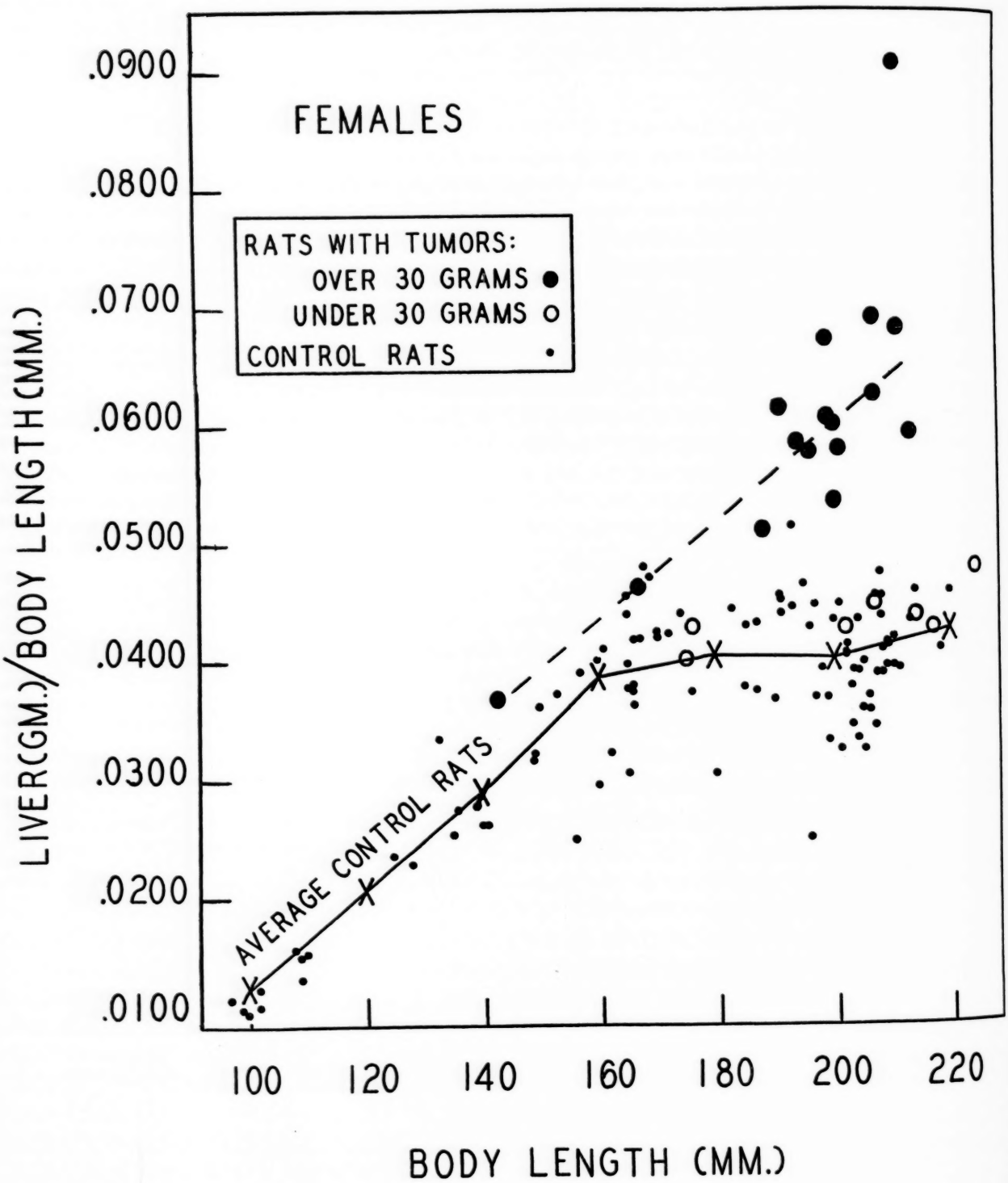

FIG. 1.—Relation of relative liver weight to the body length in females.

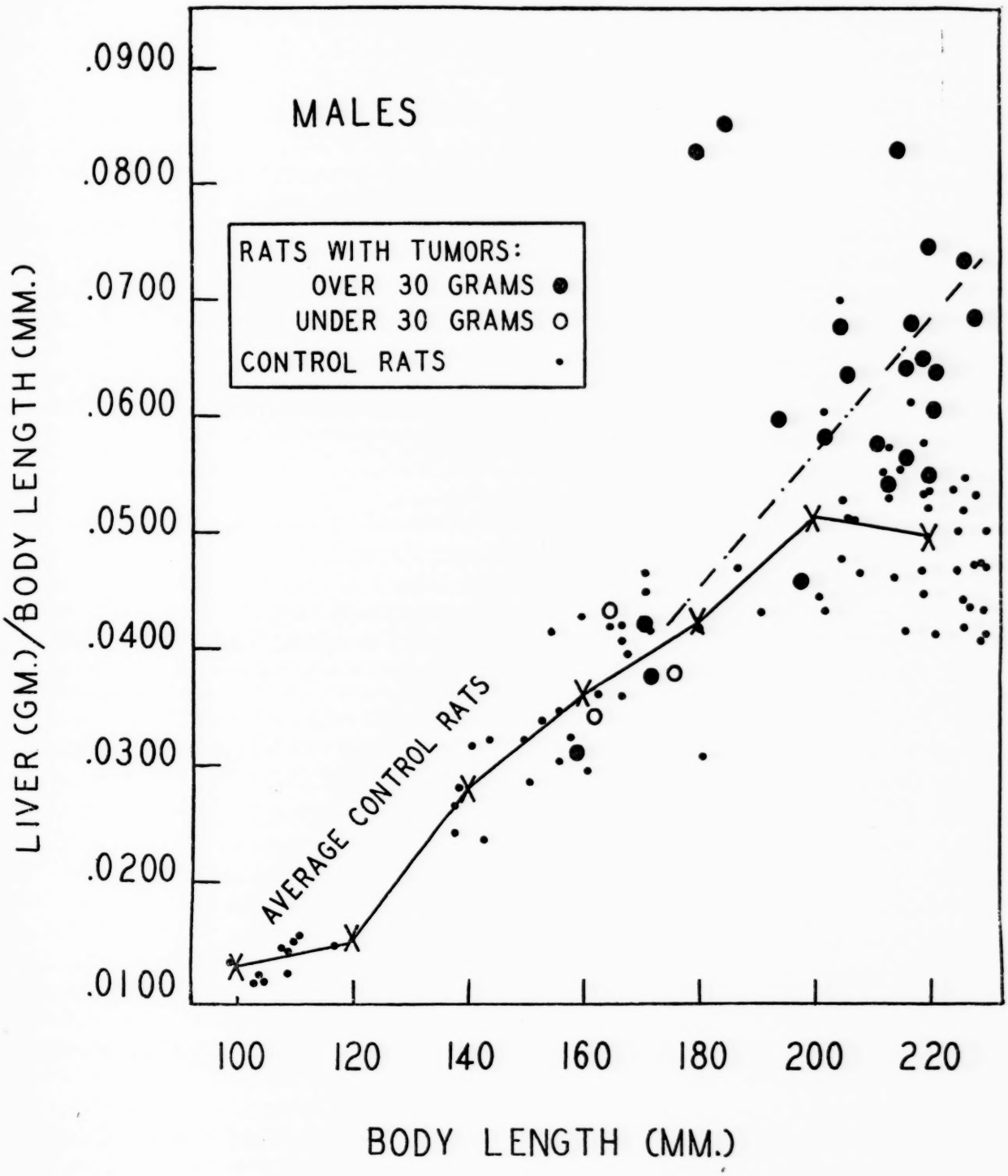

FIG. 2.—Relation of relative liver weight to the body length in males.

liver and the presence of a tumor in rats over 175 mm., although the effect on the liver was not apparent in smaller (younger) rats.

Constituents of the liver other than water may influence the weight of this organ. Glycogen and fat, as pointed out by Walter and Addis (3), represent passive reserve material, while an increase in protein content, they believe, reflects work performed by the organ. In pregnancy (2, 3), the weight of the liver as well as the percentage of total body protein in it is increased. Addis and his colleagues consider that this indicates a work hypertrophy, connected probably with protein anabolism. It is possible that similar work on the part of the liver concomitant with the synthesis of neoplastic tissue may account for the increase in the weight of the organ reported here. Moreover, the failure to find an enlargement of the liver (compared with the controls) in smaller, younger rats less than 180 mm. in body length may be explained by the supposition that these animals were still engaged in rapid body growth, so that their livers were already enlarged to the physiological maximum, and the additional stress imposed by a tumor produced no demonstrable effect upon the liver weight.

SUMMARY

1. The weight of tumor-free livers of male and female Wistar albino rats with subcutaneous growths (transplanted or primary) was compared with the weight of the tumor.

2. Neither the observed weight of the liver, nor its weight relative to the body *weight* (without the growth) was correlated with the tumor weight.

3. The weight of the organ relative to body *length* was significantly increased when the tumor weighed over 30 gm. and when the body length of the rat was over 175 mm.

4. Further growth of the tumor was not accompanied by a proportional increase in liver size.

5. The influence of a tumor on the weight of the host's liver was more evident in female rats than in males.

6. It is suggested that the enlargement of the liver was connected with protein anabolism in the growth of the tumor.

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